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Session 8a

NEW FOOD SOURCES OF KEY NUTRIENTS

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Polyunsaturated ruminant food products: a completely new range of foods derived from a more efficient animal

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ABSTRACT

The fatty acid composition of ruminant meat and dairy products can be altered by feeding a supplement rich in polyunsaturated fatty acids which have been protected against biohydrogenation. This process also increases the efficiency of the ruminant animal. The process has important implications in the fields of preventive medicine, food technology, animal husbandry and legislation. These implications are discussed, and the current state of the research programme is reviewed. The review covers some of the work being carried out in the CSIRO Divisions of Animal Physiology, Food Research and Irrigation Research, The Department of Clinical Science, Australian National University, and in the Departments of Agriculture in the States of New South Wales and Victoria. Similar and related work is being carried out in New Zealand, the United States, Norway, South Africa and the United Kingdom.

Introduction

The microorganisms in the rumen of the ruminant animal hydrogenate the polyunsaturated and monounsaturated fatty acids, and the fatty acid composition of the meat and milk of all species of ruminant animals fed a wide range of different diets is remarkedly constant (Reiser, 1951; Shorland *et al.*, 1957). The ruminant therefore is unlike monogastric animals such as the pig, chicken and man whose fatty acid composition reflects the composition of their dietary fat.

The microorganisms of the rumen can digest cellulose and allow the ruminant animal to convert feedstuffs unsuitable for most monogastric animals to meat and dairy products. The penalties for this ability to use energy sources relatively unavailable to other animals are inefficiency in the conversion of protein and lipids. The rumen microflora degrade protein and synthesize bacterial protein which may be of a lower biological value and in smaller amount than that of the original protein. Moreover the energy intake of the ruminant animal is limited by either the

bulk of the feedstuff required or by the fact that the ingestion of large quantities of dietary fat, the most concentrated form of energy, is limited. Such high levels of dietary fat lead to interference with rumen function and consequent suppression of appetite (Warner, 1960; Church, 1969; Dijkstra, 1969).

Recent developments which originated in Australia in the CSIRO Divisions of Animal Physiology and Food Research have led to the production of ruminant-derived food products with a fatty acid composition which more closely reflects that of the diet and increases the efficiency of the ruminant animal as a source of high quality human foodstuffs (Cook *et al.*, 1970; Scott *et al.*, 1970; Scott *et al.*, 1971; Scott *et al.*, 1972; Scott and Cook, 1973). These products are obtained by feeding cattle and sheep a supplement containing oilseeds, with highly unsaturated oils which have been protected against the lipolytic and hydrogenating action of the microflora of the rumen. The supplement consists of a powder composed of minute globules of sunflower seed oil clad in a skin of protein which been treated with formalin. The chemical bond between the protein and the formaldehyde is resistant to the action of the rumen microflora at the pH of the rumen (pH 6-7) but when the supplement reaches the abomasum, the true stomach of the ruminant, the protein-formaldehyde link is split by the acid conditions existing in the abomasum (Ferguson, 1967) and the protected polyunsaturated oil and the protein used for protection are available for digestion and adsorption into the tissues of the animal. Thus, these ruminants have to some extent become like monogastric animals in that the fatty acid composition of the meat (Astrup *et al.*, 1972; Cook *et al.*, 1972b; Faichney *et al.*, 1972; Hogan *et al.*, 1972; Faichney *et al.*, 1973; Newbold *et al.*, 1973; Edmondson *et al.*, 1974), and milk (Astrup *et al.*, 1972; Cook *et al.*, 1972a; Pan *et al.*, 1972; Plowman *et al.*, 1972; Bitman *et al.*, 1973; Chandler *et al.*, 1973; Gooden and Lascelles, 1973; Edmondson *et al.*, 1974) reflects that of the diet of the animal and protein utilisation is more efficient. In addition, by protecting the fat it is now possible to overcome the deleterious effects of feeding ruminants diets containing high levels of fat. This ability to biomanipulate the ruminant animal has important implications in several fields, including preventive medicine, food technology and animal husbandry.

Oil Seeds

Currently sunflower seed with its high level of linoleic acid is the major component of the protected lipid supplement used to introduce polyunsaturation into the fat of ruminant animals. A large and reliable supply of sunflower seed is a prerequisite before the polyunsaturated ruminant derived foodstuffs can make even a minor contribution to the diet of man. Unfortunately, at the present time large supplies of sunflower seed are not available. A research programme has been initiated with a view to improving the yields of sunflower seed (Muirhead, 1974). Ideally, a high yielding variety is required, the seed of which should contain high levels of oil, linoleic acid and protein. Genetic and management studies are under way in an attempt to achieve this ideal. Other potential seed sources of linoleic acid will also be investigated. Safflower seed, which has a higher content of linoleic acid than does sunflower, would be preferable, but its very hard hull does not allow its use with the current technology of supplement manufacture. It may be possible to develop a thin hulled variety of safflower seed.

Supplement Technology

Research into supplement technology is aimed at reducing the cost of supplement and improving the protection of the oil to lipolysis and

biohydrogenation. At the present time two formulations are largely in use, one based on sunflower seed with 10 % casein, and the other a mixture of sunflower seed and soya bean in a ratio of 70 : 30. The protein from the soya bean, together with that of the sunflower, eliminates the necessity for the use of casein. Both supplements are readily consumed by the animals and induce the required change in the linoleic acid level in their tissues. The choice between these two supplements or alternatives which are under investigation will depend on the cost of raw materials prevailing at the time and place of manufacture and the procedures required during processing. Pilot plants for supplement manufacture have been established in New Zealand, U. S. A. and Australia, and supplement is being made available for research.

The technology of supplements derived from fats other than those containing polyunsaturated fats is also under examination. These supplements are often referred to as "high energy" supplements, a phrase which is misleading, as the polyunsaturated supplements also provide a source of high energy content. Tallows and various other saturated or monounsaturated fats and oils are used as the basis of this type of supplement, which when fed to ruminant animals (Storry, 1974) increases the energy density of the diet, increases the butter fat content of the milk of lactating cows, may increase the growth rate of the animal, and could be of use when the animal is subjected to periods of stress.

Animal Feeding

It is probable that the ruminant meat animal will be fed the protected lipid supplements under lot feeding conditions for some weeks prior to slaughter. Dairy cattle will be fed the supplement through a complete lactation and it is hoped that the supplement will be fed in the bail at milking time. Preliminary indications are that this system will work under conditions of poor pasture, but when the pasture is lush there is difficulty in obtaining the desired level of linoleic acid. Whether this problem is biochemical in origin or due to the preference of the animal for the good grass rather than the supplement is under investigation.

Optimum conditions for feeding the animals are being established to determine the minimum period of supplement feeding and the minimum amount of supplement required to induce the required response (at least 20 % linoleic acid) in the fat of sheep, lambs and beef animals and the milk fat of lactating cows. A level of at least 20 % linoleic acid was chosen as being technologically possible and which will achieve significant reductions in the serum cholesterol levels of humans consuming polyunsaturated ruminant food products.

When considering the selection of animals for the production of polyunsaturated meat, animals with a low fat cover which have not quite reached the point of growth at which rapid fat deposition occurs are most suitable. Less time and supplement are required to induce the desired change in fatty acid composition and, in addition, the resulting meat is likely to be very acceptable to the housewife and the medical profession as it would have a lower total fat content than conventional meat.

Feeding trials have clearly shown the practical feasibility of producing polyunsaturated sheep meats within 40-50 days of feeding 350 g of protected lipid supplement per day to animals whose starting weight was 20-25 kg. Related nutritional data for beef cattle is being accumulated, but at the present time it appears as though beef animals may not be as efficient as sheep in their transfer and/or retention of linoleic acid. Beef animals fed 2000 g of supplement per day take longer than sheep to achieve the required level of linoleic acid in their fat. This

apparent difference between sheep and beef animals is under investigation. In dairy cows the percentage transfer of linoleic acid, i. e. the fraction of dietary linoleic acid which ultimately appears in the milk fat, is in the range of 20-40 %, and this figure is influenced by the nature of the basal diet, the stage of lactation and the individual animal as there is a considerable cow to cow variation.

Oxidative Stability

The increased level of linoleic acid in the food products derived from animals fed the protected polyunsaturated supplements should render them more liable to autoxidation, and this has proved to be so. Methods are being developed to overcome this problem.

Under normal Australian conditions the storage life of chilled retail cuts of polyunsaturated meats is similar to that of conventional meat and is not limited by oxidation, but, as with conventional meats, by microbial growth. The storage life of polyunsaturated frozen beef, veal (Ellis **et al.**, 1974) and sheep meats is shorter than that of conventional beef and lamb and is similar to that of pork or chickens, which contain an appreciable level of linoleic acid. The storage life is considerably extended when the meat is stored at —20° C instead of —10° C. Low storage temperatures will be essential for the long term storage of polyunsaturated meat.

The oxidative stability of polyunsaturated milk is a key factor in the commercial production and marketing of milk and other dairy products. In the initial stages of this project the supplement fed to the dairy cows was based on safflower oil and casein. The milk obtained was extremely unstable and developed undesirable oxidized flavours within 24 hours after milking, even when the milk was kept at 1° C in the dark in containers with no head space. Similar results were obtained in the U. S. A., Norway and Australia (Astrup *et al.*, 1972; Plowman *et al.*, 1972; Edmondson *et al.*, 1974; Johnson *et al.*, 1974, Sidhu *et al.*, 1975). However, when supplements based on oil seeds were used there was a considerable increase in stability and while there is a considerable cow to cow variation the average storage life under the above conditions is about six days. The storage life of conventional milk is much longer than this and it was considered necessary to devise means to improve the oxidative stability of polyunsaturated milk.

A new objective method for measurement of the oxidative stability of polyunsaturated milk was required as the conventional methods such as peroxide and thiobarbituric acid methods proved unsatisfactory. Taste panels could detect oxidized flavour in milk before any increase in these values could be detected. A method was developed based on the uptake of oxygen during the storage of milk, the oxygen level being measured by means of an oxygen electrode (Johnson *et al.*, 1974; Sidhu *et al.*, 1974). There was a good correlation between uptake of oxygen and taste panel scores. Using this method several physical techniques such as sparging with nitrogen were evaluated in an attempt to increase the storage life of milk, but while they did increase the storage life to some extent they were not altogether satisfactory. A wide range of antioxidants together with emulsifying agents were tested and several were found to be satisfactory (Sidhu *et al.*, 1974). The most effective system was the use of butylated hydroxy anisole (0.02 % of total fat) emulsified with poly oxyethylene-(20)-sorbitan monooleate, which would stabilise the milk for at least fourteen days, even in the presence of added copper. Water-dispersible formulations of β-carotene and α-tocopherol would also stabilise the milk but were not effective in the presence of added copper. One of the initiating systems of autoxidation in milk is the reaction between ascorbic acid and cupric ion

resulting in the formation of dehydro ascorbic acid and cuprous ions. Cuprous ions are one of the most potent initiators of autoxidation. Destruction of ascorbic acid with hydrogen peroxide prevented the formation of cuprous ions and led to a greater stability of the milk. If -tocopherol was then added to the milk a very stable product was obtained.

Thus by the choice of an appropriate antioxidant system, polyunsaturated milk of stability equal to conventional milk can be achieved. Experiments are under way to ascertain the reasons for the considerable cow to cow variation in oxidative stability.

Most of the polyunsaturated dairy products (Harrap, 1973) used to date have been made from milk stabilised with butylated hydroxy anisole and the storage life of the products relative to the development of oxidative off-flavours has been very good. Attempts have been made to produce dairy products without the use of antioxidants and this should be possible, at least in the case of cheese (Wong *et al.*, 1973; Czulak *et al.*, 1974). In cheese the reducing conditions created by the bacteria are not conducive to oxidation and, in addition, it is possible that the bacteria may break down any possible precursors of oxidation.

Product Development

The changed chemical and physical properties of the new types of foods allow considerable diversification of food products derived from ruminant animals (Johnson, 1974), particularly in the dairy industry, for example, the production of butter spreadable directly from the refrigerator or new varieties of cheese. It is also possible to modify the flavour of meat, particularly lamb, and to produce lamb which does not have the strong mutton odour which some potential consumers dislike. These desirable properties may be achieved at a level of polyunsaturation lower than that necessary for modifying the diet on medical grounds.

In the manufacture of butter (Buchanan *et al.*, 1970; Buchanan and Rogers, 1973; Kieseker *et al.*, 1974; Kieseker and Eustace, 1974) the churning time of polyunsaturated cream is much shorter than that for conventional cream. In order to achieve the necessary working the churning has to be performed at low temperatures (0-2° C). Vacreation of the cream at temperatures above 96° C improved the quality of the butter. A phase-inversion process has also been used to produce polyunsaturated butter. Both methods gave butter which was considered good quality first grade by taste panels, consumer panels and professional butter graders. The butters maintained grade well during storage. Polyunsaturated milk has a lower level of β-carotene than conventional milk and this led to the production of a butter with less colour than considered desirable in Australia. Addition of β-carotene not only improved the colour but also extended the storage life. Consumer reaction showed that spreadability from the refrigerator to be good and better than conventional butter., However when left at ambient temperature for some time the butter tended to oil-off.

Natural cheeses such as Cheddar, Cheedam, Gouda, Brie and Camembert have been successfully manufactured experimentally on a scale large enough to guide initial commercial manufacture (Czulak *et al.*, 1974). All varieties were found readily acceptable and contained at least 20 % linoleic acid in the fat phase. The flavour of the cheeses, whilst attractive, lacked the fullness of that of the control cheese even after many months of maturing. It has been shown (Czulak *et al.*, 1974) that the flavour of polyunsaturated Cheddar cheese can be enhanced by the addition of lipases to the milk prior to cheese manufacture and by the use of a culture of *Lactobacillus bulgaricus* in addition to the conventional cheese starter.

Cream, cheese, processed Cheddar cheese, yoghurt, icecream, cultured sour cream and milk sterilised by the ultra high temperature process have all been manufactured (Harrap, 1973) and are considered highly acceptable.

Polyunsaturated meats have been used successfully in the manufacture of smallgoods, and edible offals used to produce tallow for the manufacture of acceptable cooking margarines (Herbert and Kearney, 1974).

Flavour

The flavour of cooked polyunsaturated ruminant meats is different to that of conventional meats (Ford *et al.*, 1975; Park *et al.*, 1974). This difference is less pronounced in the case of beef than sheep meats. The flavour of the meat is blander and resembles that of chicken or pork. The cooking odours produced during the cooking of polyunsaturated sheep meat are considered undesirable by some people. The flavouring compounds thought to be responsible for the differences in flavour between polyunsaturated and conventional ruminant meats have been identified (Park *et al.*, 1974). Carbonyl compounds, principally *trans-trans*-deca-2, 4-dienal, are major components. These carbonyl compounds are those normally derived from linoleic acid during the cooking of meats such as chicken or pork which contain linoleic acid. This finding confirms the taste panel's subjective assessment that the flavour of polyunsaturated ruminant meat resembles that of chicken or pork. The volatile flavour components of meats from polyunsaturated sheep meats were characterised by the presence of much greater than normal amounts of a sweet-smelling component identified as 4-hydroxy-dodec-*cis*-6-enoic acid lactone (Park *et al.*, 1974). This lactone has also been shown to be present in much greater amounts than usual in the polyunsaturated milk fat of lactating cows (Stark and Urbach, 1974). It is thought to be responsible for the sweet and probably undesirable odour and flavour characteristics of polyunsaturated lamb fed the sunflower seed-casein supplement. However, the lactone was found to be a relatively minor and probably insignificant flavour component in the meat of lambs fed safflower oil-casein supplement and the meat and milk of polyunsaturated cattle fed the sunflower seed-casein supplement. The origins of the precursors of the lactone are under investigation.

Clinical Studies

It is now possible to produce a new range of ruminant food products of more than 20 % polyunsaturation. By replacing conventional food products from cattle and sheep these foodstuffs will permit a significant increase in poluynsaturation of ruminant animal products and thus meet one of the recommendations of expert medical committees in several countries. These committees point out that coronary heart disease is a multifactorial disease and there are many risk factors, including diet. They have come to the conclusion that in man a high intake of cholesterol and saturated fat is correlated with elevated serum cholesterol and triacylglycerol levels, both these latter apparently predisposing to a higher incidence of coronary heart disease. To date there is no conclusive proof of the direct relationships between these factors, but the Committees have recommended that certain patients at high risk should substitute foods containing polyunsaturated fats for foods containing saturated fat. The department of Clinical Science at the Australian National University, Canberra, has confirmed that diets containing polyunsaturated meat and dairy products (containing more than 20 % linoleic acid in the total lipids) in place of the conventional products, lower the blood cholesterol by 10 % and triacylglycerol levels by 15 % in man (Nestel *et al.*, 1973; Nestel *et al.*, 1974, 1975). These effects are observed within one week and are continuous over longer periods

at least up to six weeks. During the first three weeks of feeding the lowering of serum cholesterol is accompanied by enhanced faecal sterol excretion. Clinical studies are also under way in South Africa, U. S. A. and New Zealand and preliminary indications are that these studies have confirmed and extended the Australian findings.

Nutritional Evaluation

The nutritive value of polyunsaturated ruminant derived foods is under investigation in several species of animal and, to date, no deleterious effects have been observed.

Legislation

The polyunsaturated ruminant products can be properly described as a completely new class of foodstuffs and in many cases legislation applying to the conventional foodstuffs is inappropriate or may need to be altered. Examples are the legal definition of "polyunsaturated" and "animal fat" or the use of antioxidants in dairy products.

Commercialization

CSIRO has granted Dalgety Agri-Lines exclusive royalty-bearing rights to manufacture, use and sell polyunsaturated ruminant foodstuffs produced under CSIRO patent application operative in Australia and overseas. Dalgety Agri-Lines have established companies in Australia, New Zealand, the United Kingdom, Canada and the United States under the name Alta Lipids and it is likely that the products will be known as Alta products. These companies are funding research for the further development of the process and the products derived from the process.

Conclusions

Much more research is required before the real potential of the protected lipid process can be fully evaluated. There are other means of increasing the levels of linoleic acid in human diets and the consumers' choice will depend on medical advice, cost and acceptability. Even if one ignores the important health implications of the protected lipid process, the increased efficiency of the ruminant animal and the ability to biomanipulate meat and milk to vary texture and flavour will have considerable economic and technological significance.

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Recovery of rennet cheese whey in form of a children's food

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ABSTRACT

The purpose of this work was to recover whey proteins and utilise them as children's food in an acceptable form, such as are marzipans, and also to be easily stored, handled and transported to poor rural areas.

The proteins from fresh whey (pH 6.5) were heat coagulated at a pH of 4.5, adjusted with a cation exchanger. The precipitate was separated by centrifugation (laboratory experiments) or by press filtering (pilot plant experiments), air-dried at 35-40°C and used to the preparation of marzipans, with a main composition of 28 kJ and 5 g protein per unit (20 g). The marzipans had a 100 % acceptability.

The whey concentrate obtained in the laboratory experiments had a protein content of 77.3 % (N × 6.38 d.wt.) and that of the pilot plant, of 61 %. The aminogram of the two kind of experiments showed some differences but both had a high content of lysine (7.3 and 8.2 g/16 g N, respectively), and the digestibility was 91 %.

The low cost of the raw material, simplicity of the technology, appropiate nutritive properties of the marzipans make us think that the present work could provide a good possibility for elaborating a supliment food for children from poor rural areas of Mexico.

INTRODUCTION

Whey, this important byproduct of milk, is actually utilised too little. In the U. S. only the annual production of whey varies between 5 and 13 million tons, of which half of it is not utilised (Houran, 1969; Anonymous, 1971; True and Pate, 1973). If only 5 million tons of whey were used, 250.000 tons of lactose and 5.000 tons of high quality proteins could be obtained.

The composition of whey depends upon the technology followed for the processing of cheese, from which the whey results but, nevertheless, it contains 1/3 of the solids of skimmilk, that is to say: lactose, proteins, vitamins and minerals (Rosenfield, 1973).

The nutritive value of whey proteins is higher than that of all other proteins, except egg protein (Kosikowski, 1970). Its aminogram in respect with the FAO pattern is said to have a 1.35 relation (Demott, 1972), and of special interest are the high contents of lysine and sulphur-containing amino acids, which make whey proteins specially valuable as supplements to vegetable proteins of poor quality, or children's food (Forsum and Hambreus, 1972).

Besides, whey represents a serious problem of river pollution because of its biological oxygen demand (BOD) which is as high as 50.000 mg/l and so, is liable to severe legal restrictions against its discharge, "the BOD of 100 lb of whey containing the BOD equivalent of 21 persons" (Suzuki, 1972).

The purpose of this work was to recover whey proteins and utilise them as a highly nutritive food for children, in an acceptable form such are marxipans, and at the same time and easily store, handled and trasportable product, especially for rural areas.

MATERIAL AND METHODS

Fresh whey from cheddar cheese was collected about one hour after the curd was cut. This so-called "sweet whey" had an average content of 4.0 - 4.5 % lactose, 0.55 - 0.60 % proteins, 0.3 - 0.7 % fat and a pH of 6.5.

The technology followed for precipitating the proteins consisted in adjusting the pH of the whey to 4.5 - 5.0 by decationising a part of the whey with a strong acid cationic exchange resin, in the hydrogen cycle. The resin employed was a styrene polymer matrix cross-linked with 8 % divinyl benzene. The proteins were heat coagulated at 85-90° C during 20 minutes. In the laboratory experiments, where 2-3 litres of whey were processed, the precipitate was separated by centrifugation at 3500 r. p. m. during 20 min and subsequently washed with hot water, the supernatants being discarded by centrifugation. In the pilot plant experiments, 50 litres of whey provided by "Kraft Foods de México, S. A." were processed; the precipitate was separated by filtering through a filter press Columbia (made in Mexico), Modell 8, of stainless steel, using filter paper S 301 and 2 934, and subsequently washed with hot water.

The protein precipitate was dried in an aerated drying chamber at 35-40° C and homogenized with an Omni mixer.

The marzipans were prepared by mixing with other ingredientes: ground sugar, hydrogenated coco fat, flavoring substances (chocolate, strawberry, vanilla) and stabilizer (CMC food grade), and homogenized in a stainless steel homogenizer during 30 min, followed by pulverisation, dispersion, granulation and screening, so as to obtain a suitable mixture for moulding at 15 tons, then dried at ambiental temperature during 45 min and packed in cellophane bags. The main composition was 28 kj and 5 g proteins per unit (20 g).

The marzipans were tested with 200 children between 7 and 11 years of age, pupils of the primary school annexed to the "Instituto Nacional de Protección a la Infancia" (Mexico City). They were given before eating and the acceptance was 100 %, the children asking for more of those marzipans that were vanilla flavored.

The protein concentrate obtained in the various experiments was analysed for its ash content, humidity (AOAC methods), nitrogen content (Kjeldahl method) and fat (petroleum ether extract). The lactose content was calculated by difference. The aminogram was performed with an autoaminoanalyser (Phoenix Instr. Co.), the available lysine and tryptophane with colorimetric methods (Mertz *et al.*, 1964; AOAC 744, 1965; Hernandez and Bates, 1969). The digestibility of the concentrate obtained in the pilot plant experiments, serving as raw material for the marzipans, was performed with pepsine (AOAC method).

RESULTS AND DISCUSSION

Table 1 shows the composition of the protein concentrate obtained in the laboratory experiments and Table 2 that of the concentrate obtained in the pilot

TABLE 1

Composition and yield of whey protein concentrate obtained in the laboratory experiments

g/100 g protein concentrate						Protein N X 6.38 % dry wt.	Kg dry substance per 1000 L whey
No.	Humidity	N	Ether extract	Ash	Lactose		
1	7.46	11.38	13.29	0.25	8.5	77.3	5.260
2	6.50	11.44	13.37	0.28	7.0	74.6	5.000
3	7.11	10.90	22.20	0.59	0	76.3	6.241
4	4.85	10.82	15.83	0.56	8.8	72.2	5.283
5	7.57	12.16	17.20	0.98	0	83.9	5.631
6	7.02	11.57	20.0	0.39	0	79.3	5.885
Average	6.75	11.38	17.14	0.50	4.4	77.3*	5.550

* Error of the mean: ± 1.66.

TABLE 2

Composition and yield of the whey protein concentrate obtained in pilot plant experiments

No.	Humidity	N	Ether extract	Ash	Lactose	Protein N X 6.38 % dry wt.	Kg dry matter per 1000 L whey
1	10.7	7.06	24.8	1.90	17.5	50.4	4.260
2	3.0	9.90	39.6	0.10	0	66.0	–
3	8.0	9.44	36.2	0.78	0	65.2	3.360
4	11.5	8.70	37.5	1.22	0	62.5	3.700
Average	8.3	8.77	34.5	1.02	4.3	61.0*	3.760

Digestibility to pepsine (38° C; 48 h) 91 %.

* Error of the mean: ± 3.55.

plant experiments. In Table 3, the aminograms of the experiment No. 5 of the laboratory and that of a homogenous product of the pilot plant experiments, in comparison with the FAO pattern (Yoshajahu and Clifton, 1971) are shown.

The choice of rennet cheese whey of pH 6.5 as raw material was due to former investigations (Ionescu *et al.*, 1967; Racotta, 1972; Racotta *et al.*, 1971), concording with other publications (True and Pate, 1973), which demonstrated that the initial acidity of the starting material had a detrimental effect upon whey protein coagulation. The partially decationising reduces the divalent cations to a ratio cations: proteins of 1 : 35 parts b. wt. (Wingerd, 1972) and the pH to 1.5 - 2.0. The subsequent rapid adjustment of the total whey to a pH of 4.5 - 5.0, followed by heating at 85-90° C was in accord with personal investigations and other pulished data, so as to obtain the maximum recovery experiments was 77 % and that of the pilot plant experiments was 42 %, the dry matter yields being 0.55 and 0.38 %, respectively. The coagulation was checked at different temperatures, and at the

TABLE 3

Aminogram of the protein concentrate obtained from whey

Amino acid	g/16 g N		
	Laboratory experiment	Pilot plant experiment	FAO pattern
Lysine	8.2	7.3	4.2
Histidine	–	14.6	–
Arginine	6.1	5.9	–
Aspartic acid	11.1	6.6	–
Treonine	5.9	2.0	2.8
Serine	2.9	2.9	–
Glutamic acid	15.3	8.4	–
Proline	7.4	5.4	–
Glicine	1.9	1.2	–
Alanine	3.9	2.3	–
Cistine	2.7	0.8	–
Valine	1.9	1.1	4.2
Methionine	2.2	2.6	2.2
Isoleucine	5.2	2.0	4.2
Leucine	12.7	12.0	4.8
Tyrosine	5.3	1.4	–
Phenylalanine	5.8	1.4	2.8
Tryptophane	0.5	0.4	1.4
Available lysine	–	3.9	–

above mentioned temperature the best protein precipitation, in great floculus levaing a clear supernatant, was obtained.

The subsequent rapid centrifugation (in laboratory experiments) followed by thorough washing tried to eliminate lactose as much as possible, so as to prevent the obstruction of available lysine by Maillard reaction, and eliminate as much as possible the lactose from the protein concentrate.

From Table 1 it can be seen that in the laboratory experiments the lactose content in the product was in an average of 4.4 g/100 g concentrate. In the four pilot plant experiments all but one had no content of lactose. In fact, in the experiment in which 17.5 g lactose/100 g concentrate were present the filtration occurred some hours after, because of some technical difficulties. This accident demonstrates once more that during this time lactose was incorporated into the precipitate and could not be eliminated sufficiently by washing. That is to say that in the technical process the separation of the protein floculus should occur as rapidly as possible.

In the laboratory experiments a protein concentrate with an average content of 77.2 % of proteins (N x 6.38 d. wt.) was obtained, while in the other experiments the protein concentration was about 60 %, fact that has to be related with little differences in the technological process and also with the different composition of the starting material. For the laboratory experiments the starting material consisted of whey obtained from pasteurized, homogenized skimmilk, while for the pilot plant experiments the whey furnished by "Kraft Foods" was obtained from natural milk.

The higher fat content (24-37 %) in the pilot plant concentrate, in comparison to the average content of 17 % in the laboratory obtained concentrate is also demonstrating the same statement.

Digestibility was analysed only for the concentrate that served as starting material for the marzipans and, as it is shown in Table 2, was 91 %.

Table 3 shows the results of the aminograms of one of the laboratory obtained concentrates (experiment No. 5), which had the highest protein content, in comparison with the aminogram of the starting material for the marzipans and the FAO pattern. Available lysine was determined only for the starting material for the marzipans.

As it can be seen, the aminograms of the two concentrates were different, but both showed a high content of lysine (173-195 % of the FAO pattern), from which almost half of it was in its available form. In comparison with the FAO pattern, the concentrate obtained in the laboratoy experiments showed a better agreement, with defficiencies only for valine and tryptophane. These facts are showing the dependence of the amino acid pattern from the technology employed and the existence of the possibility of improvement without great technical modifications. Tryptophane being the most susceptible to oxidation, the use of another method of drying with less exposure to air (spry drying, for example) and also hermetical conditions of packing and storage, could make us hope for a better proportion of this special amino acid in the final product.

It is convenient to make clear that the present process was intented to be the cheapest possible. It is also important to note that in Mexico there are defficient nutritional areas, lacking also communications, refrigeration and storage facilities. Thus, to reach them with effective help, it is necessary to have a simple product that does not deed special conditions for storage and handling and has easy acceptance. Marzipans proved to be very well accepted by children. In these poor rual areas the principal source of protein is corn, defficient in lysine, methionine and tryptophane; the helpfull product should mostly compensate these defficiencies. From what one can see, by increasing tryptophane content —which is possible because the whey contains 128 % of the FAO requierement (Demott, 1972)— the present product could conveniently be used as a food supplement for these areas.

CONCLUSION

Good acceptability, appropiate nutritive properties of the protein, low cost of the raw material, simplicity of the technology make us think that the present work could provide a good possibility both for recovering a valuable product, actually not sufficiently employed, and elaborating a good supplement food for poor rural areas in Mexico.

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Fortification of Milssal as a potential vehicle of providing lysine in Korean food system

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ABSTRACT

Milssal is a polished, partially gelatinized and pressed wheat grain and it is consumed in Korea. The potential use of Milssal as a vehicle of providing lysine in the Korean food system has been examined since lysine is the most limiting amino acid in the Korean diet. Fortification of Milssal with lysine can be achieved easily at the manufacturing site of Milssal by spraying L-lysine • HCl solution, instead of water, to the cleaned and debranned wheat grains during the regular wetting process. This process is technically feasible and economically attractive. No difference in appearence, taste and storage stability was found between Milssal before and after lysine fortification, assuring the existing acceptability of the fortified product.

INTRODUCTION

Protein deficiency is one of the nutritional problem in Korea as seen in other developing countries. A recent study indicated that at least 61 % of urban Korean families suffer from some degree of protein insufficiency (Cantor *et al.*, 1971). The problem arises mainly from the inadequate food distribution and the low biological value of the dietary proteins. Although the total per capita daily supply of calories, protein, and fat has steadily increased in the past decade, Koreans heavily rely on vegetable sources for the supply of nutrients; that is, 94 % of total calories and 80 % of protein are from vegetable origin (FAO Korean Association, 1972). In fact, clinical observations have shown some degree of chronic deficiencies in protein and vitamins (Kang and Kim, 1968; Haw *et al.*, 1970). This suggests that not only quantitative improvement of over-all food supply, but also qualitative improvement of protein in the Korean diet is urgently needed.

Rice and barley are the main stay of meals served 3 times a day along with soup and other accessory side dishes. Wheat is consumed so far mainly in the form of bread and noodles and increasingly replacing rice and barley. In 1972 per capita daily supply from the food balance sheet of rice, barley and wheat was 361 g, 160 g, and 84 g, respectively. Due to the fact that lysine is the first limiting amino acid for these cereals, it is likely that lysine is the most limiting in the Korean diet. If this is

the case, fortification of the Korean food system with lysine should be considered as one of the practical means of improving the protein quality of Korean diet. Under the present milling and distribution systems of rice and barley in Korea, however, fortification of such crops is not practical. In particular, washing loss of fortified lysine before cooking hinders the fortification from being useful.

Meanwhile, it is of interest to observe that wheat is being consumed popularly in the form of Missal in Korea. Milssal is a polished, partially gelatinized and pressed wheat grain. Mainly due to economical reasons, today many Korean households stretch their rice by mixing with Milssal up to 20 %. The production of Milssal started in 1972 by a process covered by Korean patent 3426 and amounts to about 60,000 tons per year. Since Milssal is prewashed, it can be added without washing to the washed rice prior to cooking. Thus, if lysine can be fortified, Milssal may serve as an ideal vehicle for providing lysine in the Korean food system. Milssal is packed and sold in 3 or 5 Kg size bags and is readily available to consumers. The proximate composition of Milssal is given in Table 1 and its amino acid composition is compared with the provisional amino acid scoring pattern (FAO, 1973) in Table 2. The first limiting amino acid of Milssal is lysine, and the second threonine.

The present study deals with mainly estimation of the optimal level for lysine fortification and development of a practical procedure for Milssal to be fortified.

TABLE 1

Proximate analysis of Milssal

Water (%)	12.9	Iron (mg %)	3
Protein (%)	11.1	Phosphorus (mg %)	180
Fat (%)	1.2	Vitamin B_1 (ug %)	200
Ash (%)	0.9	Vitamin B_2 (ug %)	50
Calcium (mg %)	20.0	Niacin (ug %)	1,200

TABLE 2

Amino acid composition of Milssal

	Milssal (mg/gN)	FAO Provisional Pattern (1973) (mg/gN)
Isoleucine	212	250
Leucine	440	440
Lysine	148	340
Methionine + Cystine	208	220
Phenylalanine + Tyrosine	463	380
Threonine	166	250
Tryptophan	69	60
Valine	321	310

EXPERIMENTAL

1. Estimation of lysine fortification level for Milssal

If Milssal is fortified with lysine to the level of the second limiting amino acid of Milssal, the quantify of lysine to be added can be calculated easily from the data shown in Table 2. Furthermore, if

the needed lysine is to be supplied for Korean diet by way of lysine fortification of Milssal, it is essential to quantify the amount of lysine in the diet. In this study the food consumption data were obtained from the national nutrition survey report (Haw *et al.*, 1970). The nutrition survey was conducted between July 29, 1969 and August 15, 1969 covering 952 households and 6,218 individuals inhabiting in cities, farming villages, fishing villages, mining villages and mountinous villages. Per capita consumption of amino acids are calculated using the national average data on protein intake (Haw *et al.*, 1970), nitrogen conversion factor (FAO, 1973) and data on the amino acid composition (FAO, 1970; Chai *et al.*, 1961). In this way, amino acid composition of Korean diet is compared with FAO provisional amino acid scoring pattern (FAO, 1973) to calculate the lysine gap between the two systems and to figure out the differences in lysine and threonine.

2. Development of lysine fortification process

Wheat used in this study was obtained from a Milssal manufacturing company near Seoul and has the following chemical composition; moisture, 11.1 %; crude protein, 12.7 %; crude fat, 1.5 % and ash, 1.4 %. Food grade L-lysine·HCl (98 % above) was obtained from Miwon Co. Ltd (Busan, Korea).

Actual fortification study was conducted at the manufacturing site utilizing the existing facilities for Milssal manufacturing. Production of Milssal is illustrated by the flow sheet in Fig. 1. Briefly, dry cleaned wheat is debranned through the first and second debranning processes, and then sprayed with water to moisten the wheat grain to around 18 %. After passing through three consecutive steaming processes at 120° C for 2 minutes each, wheat grain is conditioned at 160° C for 30 seconds and partially pressed in order to give more cracks on the surface of the grain which in turn allows quick water absportion during cooking. The pressed grain is dried to a water content of 13 % by passing through a fluidized bed dryer at 70-75° C for 1-2 minutes, followed by cooling, sieving and packing processes to give the final product. For lysine fortification, a solution of L-lysine·HCl was sprayed instead of water during the wetting process above mentioned. For example, in order to fortify Milssal with lysine to the level of 0.12 %, 1.23 %, a lysine solution containing 6.14 Kg of L-lysine·HCl in 500 l of water was sprayed for 45 minutes by the rate of 8.3 l/min. to the wheat grains which are passing through the wetting process at the rate of 83.3 Kg/min. during Milssal production. At 2 minute intervals batches of 200 g Milssal were taken out for 75 minutes, including 15 minutes each before and after fortification, and the lysine content in the samples was analyzed using a method developed by Ferrer *et al.* (1969). In this particular Milssal manufacturing line, 5 tons of wheat are processed and 500 l of water is required for 1 hour operation.

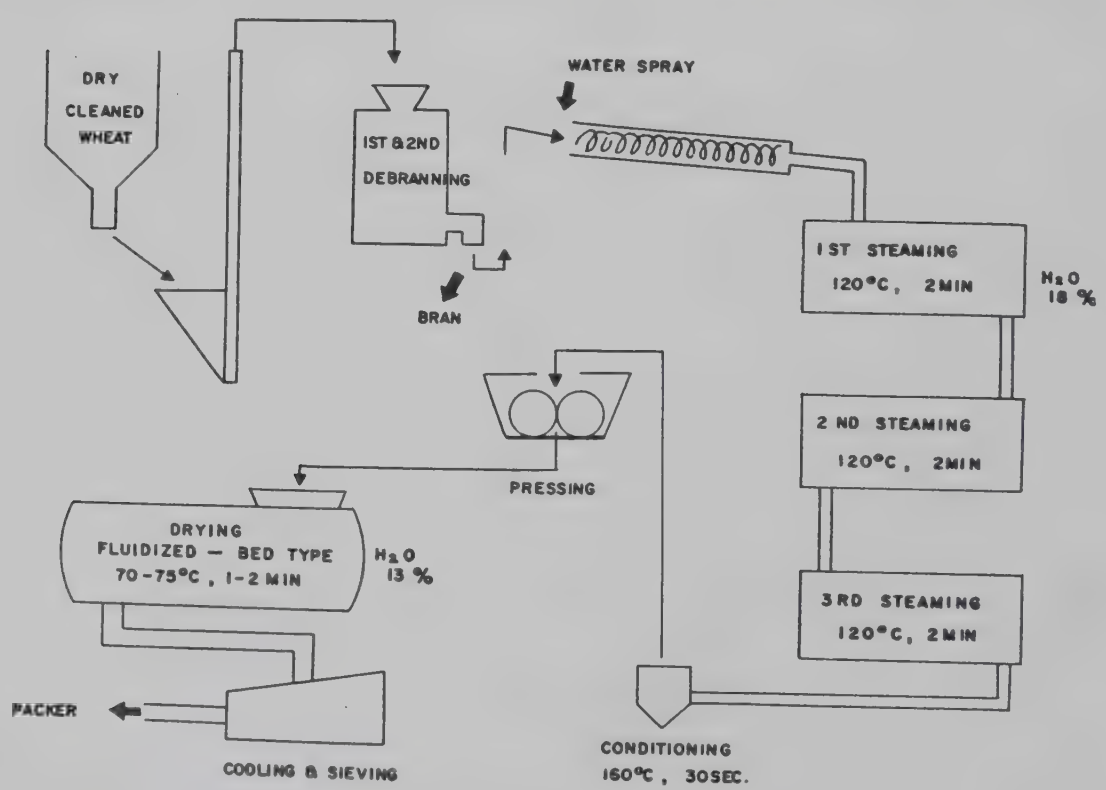


Fig. 1.–Flow sheet for the production of milssal.

RESULTS AND DISCUSSION

1. Estimation of lysine fortification level for Milssal

It has been reported that the nutritional quality of wheat protein can be enhenced by fortification with lysine in the ratio of 71.1 mg per grams nitrogen of wheat to the level of fish protein (Kyowa Hakko Co., 1970). It was also reported that PER of wheat flour was increased from 0.65 to 1.56, by fortification with 0.2 % L-lysine·HCl, and, in case of whole wheat, the PER was increased to 1.33 and to 1.45 by fortification with 0.1 % and 0.2 % lysine levels, respectively. Other report indicated that fortification with L-lysine·HCl above 0.2 % level did not improve the PER significantly over that of 0.2 % lysine fortified wheat flour (Howe *et al.*, 1965).

When amino acid composition of Milssal is compared with the FAO provisional amino acid scoring pattern, as shown in Table 2, the calculated amino acid score of Milssal is 43.5 with lysine being the first limiting and 66.4 with threonine being the second limiting amino acid. The amount of lysine needed is thus calculated to be 0.18 % L-lysine·HCl per 100 g of Milssal in order to increase amino acid score of Milssal from 43.5 to 66.4. During the fortification procedure, however, about 10 % of the added fortificant is lost. Thus, if one considers another 5 % as the safety factor, actual amount of L-lysine·HCl to be added in the Milssal manufacturing process should be 212.7 mg per 100 g of Milssal.

However, as pointed out earlier, Milssal is not consumed as such but as a mixture with rice, which is cooked and consumed along with other accessory side dishes. Since lysine is also the limiting amino acid in rice, an additional amount of lysine is needed to supplement the portion that is lacking in rice. However, some extra lysine may be supplied from the side dishes, such as, soybean products, meats, and fishes. Amino acid compositions of the Korean diet are tabulated in Table 3 from available data on nutrition survey (Haw *et al.*, 1970) and compared again with FAO provisional scoring pattern (FAO, 1973). As expected, lysine is identified as the most limiting amino acid in Korean diet with an amino acid score of 67.4. The second limiting amino acid is threonine with an amino acid score of 78.2.

TABLE 3

Amino acid composition of Korean diet

	Korean diet (mg/gN)	FAO Provisional Pattern (1973) (mg/gN)
Isoleucine.	244	250
Leucine.	464	440
Lysine.	229	340
Methionine + Cystine.	223	220
Phenylalanine + Tyrosine. ..	478	380
Threonine.	195	250
Tryptophan.	83	60
Valine.	305	310

On the other hand, the average protein intake is identified from the same nutrition survey as 65.68 g per day per person. When we take edible food waste (10 %), digestibility (80 %) and amino acid score (67.4) into account, the actual utilizable protein intake becomes 31.9 g. This value is significantly lower than 37 g which is the safe level of protein intake suggested by FAO (1973). The average

intake of lysine is calculated as 2,535 mg per day per person utilizing data from the same nutrition survey (Haw et al., 1970). This figure is much lower than the values for USA and Japan, i. e., 5,200 mg and 4,523 mg, respectively (Kondo, 1960; Isobe *et al.*, 1966). Furthermore, if the Lörstad's 20 % deficiency rate is applied to the above average figures of protein and lysine intakes, the expected actual intakes of the nutrients in low income groups become much more insufficient. This situation appears as the cause of the protein deficiency observed in Korea (Kang and Kim, 1968; Haw *et al.*, 1970).

Since the amount of rice and barley consumed is 450 g per person per day (Haw *et al.*, 1970), 20 % replacement would require 90 g Milssal. Further improvement of the amino acid score of Korean diet from 67.4 to 75 will require an additional 38 mg of lysine for every gram of nitrogen and thus 432.4 mg of lysine for 11.35 g of total nitrogen in Korean diet. This is equivalent to 480.4 mg % of lysine (600.3 mg % L-lysine·HCl) for Milssal. If 10 % loss and 5 % safety factor are considered, the amount of L-lysine·HCl needed for the fortification will be 700 mg per 100 g of Milssal. When 90 g of such fortified Milssal is used in the Korean diet, the per capita daily intake of lysine would become 2,894 mg and the amino acid score of the diet 75.

2. Development of the fortification procedure

During the process of converting polished wheat into Milssal, the average product recovery is 96.7 %. A typical result of lysine fortification to the level of 0.12 % at the Milssal manufacturing site is illustrated in Fig. 2. The amount of lysine incorporated into Milssal gradually increased to a level of plateau and then decreased. At the plateau the amount of lysine incorporated was 0.117 ±0.009 % and the loss of lysine about 10 %. In this manner, it is possible to maintain the amount of lysine to be added by adjusting either the concentration of lysine or the flow rate of a lysine solution. The loss of lysine is due to cracked wheat (4.2 %), dusts (1.2 %) and other reasons. Also the loss of lysine through equipment contact during batch-type fortification process comprises a significant portion of the total loss but this loss can be reduced substantially for a continuous operation. The 10 % loss of lysine is quite smaller than that reported for the fortification of bulgur wheat with lysine (Ferrel et al., 1972) which amounts to 24 %.

There was no difference in appearence, taste and storage stability of Milssal before and after fortification (Cheigh et al., 1974). Since the present study indicates

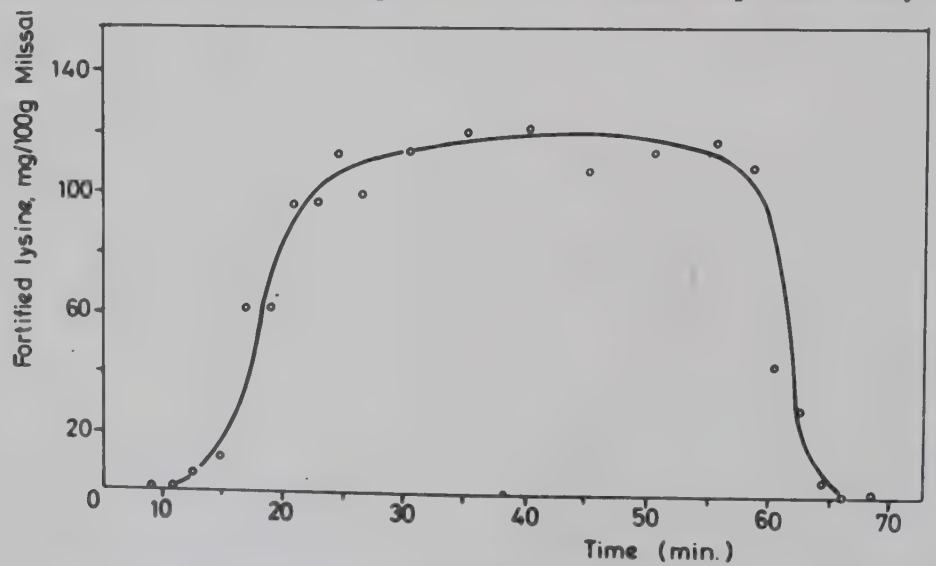


Fig. 2.–Mode of lysine incorporation during the fortification process.

that the fortification can be achieved satisfactorily by utilizing the existing Milssal manufacturing facilities without additional equipment and facilities, the cost of fortification could be down to the level of the price of lysine. If one assumes the price of lysine as $3.00 per Kg, the relative cost increase due to the fortification with 0.2 % and 0.7 % L-lysine·HCl would be 4 % and 15 %, respectively.

If such a fortification program is adopted for commercialization and the fortified product is accepted by consumers, the nutritional impact must be immense. Some anticipated effects are tabulated in Table 4. The utilizable protein of Milssal will be increased from 4.9 g to 7.5 g by 0.2 % L-lysine·HCl fortification which is equivalent to an increase of 2.6 g per 100 g Milssal. Furthermore, consumption of 90 g of 0.7 % fortified Milssal as a part of 450 g of rice and barley will increase the utilizable protein from 44.2 g to 50.7 g, which is equivalent to 6.5 g increase in protein intake per day per person.

TABLE 4

Effect of lysine fortification on the utilizable protein

	Protein content	Utilizable protein	Increase
Milssal.	11.1g/100g	4.9g/100g	–
Milssal + 0.2 % L-lysine . HCl.	11.3g/100g	7.5g/100g	2.6g
Per capita daily intake.	65.7g	44.2g	–
Per capita daily after fortification. ...	67.6g	50.7g	6.5g

Today, self-sufficiency of food supply in Korea is about 70 % and, among others, the limited arable land is one of the major factors responsible for this situation. Since lysine can be produced economically by fermentation without using land it should offer many benefits to improve the quality of Korean diet and thus eventually should contribute to the nutrition of Korea. It is fortunate for Korea to have her own commercial production of lysine since 1973.

ACKNOWLEDGEMENT

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Production and properties of a nutritious beverage base from soy products and cheese whey

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ABSTRACT

Fluid sweet cheese whey, full fat soy flour, soybean oil, and corn syrup solids, when combined, pasteurized, homogenized, condensed *in vacuo* to over 40 % total solids and spray dried yield a freeflowing powder, readily reconstitutible with water to form a nutritious beverage with high protein content. A vitamin-mineral premix may be dry blended with the powder to increase the nutritional quality further. The formulation of the product is flexible and the taste quality is bland. The product shows good storage life and resists oxidative change in the dry state.

INTRODUCTION

Milk production in developing countries has often been inadequate to meet the nutritional need of the population. For many years commodities and processed foods, including nonfat dry milk, have been purchased by the U. S. Department of Agriculture (USDA) for distribution to needy people abroad by the U. S. Agency for International Development (AID) through the United States Voluntary Agencies, through government-to-government bilateral agreements, and through contributions to the World Food Program of the Food and Agricultural Organization of the United Nations (Johnson, 1973). Over 100 million people in about 90 developing countries have participated in these programs (Senti, 1974).

Due to the steady decline in milk production, nonfat dry milk is in short supply and priced out of reach for overseas distribution in U. S. Food-For-Peace programs (Anonymous, 1974). In late spring of 1973, a joint USDA-AID effort was begun to develop a nutritious beverage powder mix specifically formulated as a

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Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

dietary supplement for preschool children receiving inadequate protein. It was not intended to serve as the sole source of food. When reconstituted with water, the beverage was to supply all the nutrients of whole milk; it was also to be cheaper than nonfat dry milk to produce and ship overseas.

The work reported here describes the production and properties of a new whey-soy beverage powder designed to serve as a nonfat dry milk replacer. The storage stability characteristics of the powder as determined by flavor and chemical measurements are also discussed.

MATERIALS AND METHODS

Ingredients

Food grade full fat soy flour containing 42.7 % protein, 20.2 % fat and 4.4 % moisture was used in the preparation of the product. The soybean oil used was refined, partially hydrogenated and winterized. It also contained methyl silicone, polysorbate 80, polyglycerides and the antioxidants butylated hydroxy toluene (BHT) and butylated hydroxy anisole (BHA). Sweet whey used for the prototype was fluid pasteurized Mozzarella whey preconcentrated at the cheese plant to 16 % total solids (TS). The corn syrup solids employed for the industrially produced prototype were a 42 DE product. Commercially prepared vitamin and mineral premixes were used to fortify the whey-soy beverage mix.

Production Methods

Over 4.000 kilos of a commercial prototype for the whey-soy beverage powder were prepared by wet blending full fat soy flour, corn syrup solids and soybean oil into fluid sweet whey in a stainless steel tank equipped with a turbine agitator. The formulation used for production is shown in Table 1. The wet blend, heated to 38-43° C, was homogenized in a Manton-Gaulin 1135.5 liter per hour homogenizer in two stages using pressures of 175.8 kg/cm^2 and 38.7 kg/cm^2. The mixture was then pasteurized by a high temperature short time procedure at 77° C for 15 seconds and condensed to 47 % TS in a single stage down blast evaporator rated at 454 kilos of water/hour evaporation. Preheat temperature before evaporation was 77° C and pump out temperature 38-43° C. The concentrate was spray dried using a 4.2 meter diameter anhydrous PEEBLES—TYPE DRYER: The inlet air temperature was 149° C and outlet temperature 93.3° C. Powder outlet temperature was 49° C. 182 Kilo lots of the cooled powder were dry blended with 1 % by weight of the vitamin-mineral premixes using a 0.57 cubic meter PK twin shell blender operated for 10 minutes. A flow diagram of the procedure is shown in Figure 1.

Packaging and Storage Conditions

For storage stability studies, samples of the fortified and unfortified spray dried beverage powders were packed in No. 1 cans in a nitrogen-hydrogen mixture (95 : 5) and stored at —18° C. An oxygen scavenging catalyst was included to remove residual oxygen. These samples served as controls. Additional samples of the two powders were air packed in 2 mil thick polyethylene bags which were heat sealed. These samples were stored in constant temperature incubators set at 25 and 43° C.

Organoleptic Tests

Withdrawals from storage at all temperatures were made after 1, 2, 4, 8, 12, 18, 20, and 24 weeks. The samples were reconstituted to 15 % TS just prior to tasting. Coded randomized samples were presented to a panel of experienced dairy product judges and rated by using the nine point hedonic scale devised by Peryam and Pilgrim (1957). The panel was also asked to identify specific flavors and rate their intensity on a scale of 0-4. A samples score sheet is shown in Figure 2. Flavor intensity values (FIV) for oxidized and rancid flavor cirticisms were calculated from this score sheet by computing the total intensity score for each flavor and dividing by the total number of judges.

Statistical evaluations for significance were made on the experimental data after completion of each taste panel. Analysis of variance and Duncan's Multiple Range Tests according to Larmond (1970) were used to obtain results at each storage interval.

Analytical and Physical Tests

Proximate compositional analysis was carried out on the unfortified commercial prototype by a commercial laboratory. Procedures used for the determination of total nitrogen, fat, moisture, and ash in the whey-soy beverage powder were those recommended by the AOAC (1970). Carbohydrate was

TABLE 1

Formulation of unfortified whey-soy beverage powder

Ingredient	Percent
Sweet whey solids.	41.7
Full fat soy flour.	36.9
Soybean oil.	12.3
Corn syrup solids.	9.1

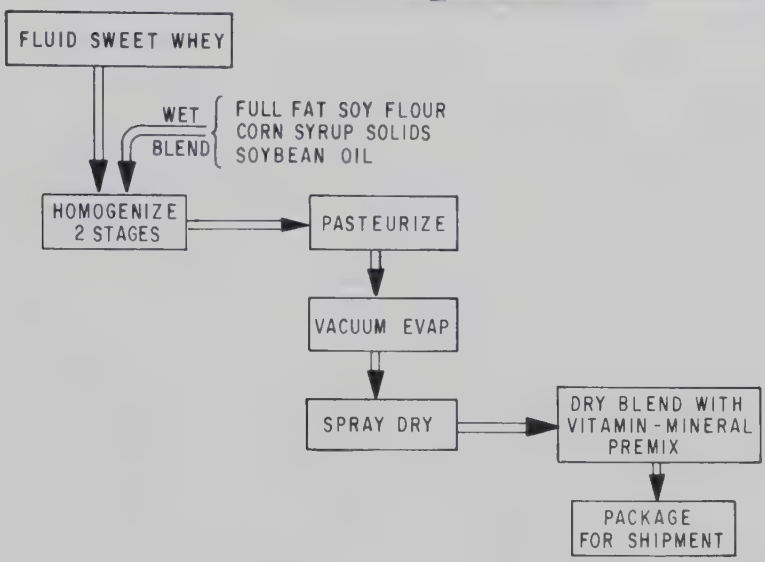


Fig. 1.–Production of whey-soy beverage powder.

SCALE

0 = NONE
1 = QUESTIONABLE
2 = SLIGHT
3 = DISTINCT
4 = STRONG

Please use one of the above numbers to describe the intensity of each flavor listed below

FLAVOR CRITICISM	SAMPLE CODE								
COOKED									
FEED									
RANCID									
STALE									
OXIDIZED									
OTHER									

Fig. 2.–Intensity score sheet used for evaluation of whey-soy beverage powder.

determined by difference and caloric content by calculation. Dispersibility of the dry samples was measured by a modification of the method of Stone *et al.* (1954) as developed by Kontson *et al.* (1965). sinkability by the procedure of Bullock and Winder (1960) and solubility index and bulk density by methods recommended by the American Dry Milk Institute (1947).

Peroxide values were determined on all stored samples as another measure of oxidative stability. Fat for peroxide analysis was extracted from the powders without the use of heat by blending the dry

samples with Celite 545 (1 : 2). The dry mixtures were placed in glass columns, 30 cm x 1 cm I. D., equipped with coarse sintered glass discs, and peroxide-containing fat was eluted into 25 ml volumetric flasks by using a benzene: methanol mixture (70 : 30). After appropriate dilution of the samples with the same solvent mixture, peroxide values were determined directly by the colorimetric procedure of Hills and Thiele as described by Stine *et al.* (1954).

Animal Feeding Studies

Animal feeding studies were conducted on the unfortified prototype by a commercial laboratory. The protein efficiency ratio (PER) assay was conducted according to an AOAC procedure. The sample was compared to ANRC reference casein. Protein evaluation, using the net protein utilization (NPU) method was conducted according to the procedure of Miller and Bender (1955).

RESULTS AND DISCUSSION

Of prime importance in the development of the new nonfat dry milk replacer was the decision to use whey in the formulation. Research activities carried out by USDA's Dairy Products Laboratory (DPL) had already shown that sweet whey-soy flour mixtures could be spray dried using conventional dairy plant equipment to produce powders readily reconstitutible with water to yield milk-like beverages (Guy *et al.*, 1969). Because whey contains levels of sodium and potassium considered to be too high for ingestion by very young children, additional pilot plant runs were made at DPL in order to gain information about how the new product should be formulated with whey to meet the nutritional needs of preschool children.

Edible oil added to the formulation not only served as a diluent to aid in reducing the osmotic activity of water in the reconstituted beverage, but also helped satisfy the requirement that the new whey-soy drink should be a significant source of calories. In order to reduce the osmolality of the reconstituted beverage still further and, at the same time, supply a sweetening effect to reduce soy flavor, a high molecular weight material, 42DE corn syrup solids, was included in the formulation. Inclusion of these two ingredientes reduced the salt concentration of the whey by 20 % in the finished powder.

Experimental whey-soy flour formulations containing additional soybean oil and significant amounts of corn syrup solids were sucessfully spray dried in the DPL pilot plant using the techniques developed by Guy *et al.* (1969). There were no changes in solubility and dispersibility of the dried powder upon reconstitution. In addition, organoleptic evaluation of the reconstituted products showed reduced levels of soy flavor in the new formulations.

On the basis of these results, the ARS whey-soy formulations were modified further by nutritionists to meet AID requirements. An industrially prepared prototype, financed by AID under a Participating Agencies Service Agreement with USDA's Economic Research Service (ERS) was produced for evaluation using the formulation shown in Table 1. The primary objective was met in that the product contains a significant amount of sweet whey solids. Most of the protein is contributed by the soy flour. The proximate composition of the new beverage powder is shown in Table 2. One per cent vitamins and minerals were added to the powder for testing. The composition of the vitamin-mineral mix is shown in Table 3.

Another requirement of the new beverage powder was that its PER, based on animal feeding studies, should not be less than 1.8 and preferably higher. The industry prototype had a corrected PER of 2.1 compared to reference casein of 2.5. The NPU value was 75 compared to a value of 84 for casein.

The beverage powder was also required to be easily reconstitutible in water. Physical properties related to reconstitubility are shown in Table 4. The slight

TABLE 2

Proximate composition of unfortified whey-soy beverage powder

Component	Percent
Protein (total N X 6.25).	21.2
Moisture.	3.9
Ash.	5.1
Fat.	21.4
Fiber.	0.8
Carbohydrate.	47.6
Calories/100 g dry powder.	468

TABLE 3

Composition of vitamin-mineral premix added to whey-soy beverage powder

Vitamins added	Amount/100 g dry powder
	I.U.
Vitamin A.	1930
Vitamin D.	233
Vitamin E.	8.72
	mg
Thiamin.	.32
Riboflavin.	.45
Niacin.	5.78
Vitamin B_6.	.19
Vitamin B_{12}.	.005
Calcium pantothenate.	3.21
Folacin.	.23
Ascorbic Acid.	46.8
Minerals added	
Calcium.	280
Iron.	17.6
Iodine.	.05
Zinc.	1.05

TABLE 4

Physical properties of whey-soy beverage powder

Test	Unfortified	Fortified
Solubility index.	4.0 ml	4.3 ml
Dispersibility.	89.3 %	81.7 %
Sinkability.	69.7 %	81.8 %
Bulk density		
Untapped.	.48 g/cc	.45 g/cc
Tapped.	.61 g/cc	.67 g/cc

change in solubility index of the product fortified with vitamins and minerals over the unfortified product, probably reflects the presence of the slowly dissolving mineral salts added. Although fortification somewhat reduced the dispersibility of the spray dried powder, the sinkability, another measure of rate of powder hydration, was increased, so the overall solubility was not noticeably affected when the powder was reconstituted for tasting.

Trials carried out in the DPL pilot plant showed that foam-spray drying, used as a method of making readily dispersible nonfat dry milk (Bell *et al.*, 1963), improved the dispersibility of the whey-soy beverage powder; however, there was a corresponding reduction in bulk density.

The new beverage powder was also required to have good storage stability characteristics. To aid in assuring good oil stability and good initial flavor, highly refined, partially hydrogenated soybean oil containing added antioxidants was used in the formulations. Organoleptic evaluation and determination of peroxide values during 6 months' storage at three different temperatures were used as two measures of oxidative stability of the whey-soy beverage powder.

Samples were reconstituted for organoleptic evaluation within 1 hour of the time set for the panel. Long standing after reconstitution promoted the development of oxidized flavor in the sample fortified with vitamins and minerals, particularly if an electric blender was used for mixing.

Table 5 shows hedonic flavor rating of the whey-soy beverage powder stored at three different temperatures for various times. The unfortified sample stored at 43° C showed a significantly lower score from its control after 8 weeks of storage. The fortified sample stored at 43° C showed a significantly lower score only after 18 weeks of storage. None of the samples stored at —18° C and 25° C showed any significant change in flavor score over the 6 month storage period. The hedonic flavor scores shown in Table 5 for the fortified sample tended to be lower at all temperatures and storage times than the scores for the unfortified powder. Examination of the intensity values for off-flavors detected by the judges indicated

TABLE 5

Average hedonic flavor scores of samples of whey-soy beverage powder stored at three different temperatures

Storage temperature (°C)	Time of storage - weeks								
	0	1	2	4	8	12	18	20	24
					Hedonic rating				
					Fortified				
–18.		3.7	4.7	3.8	3.3	4.3	5.3	5.3	4.8
25.	4.5	3.3	4.4	3.8	3.8	4.3	4.9	4.0	4.7
43.		4.1	4.3	3.3	3.0	3.3	3.1*	3.2*	2.4*
					Unfortified				
–18.		4.9	4.6	4.7	5.0	5.0	5.7	5.3	6.0
25.	5.0	5.1	4.4	4.5	4.5	4.3	4.9	5.5	5.7
43.		4.4	4.3	4.0	3.5*	3.3*	3.7*	3.0*	2.0*

*** Significantly different from the control at the 1 % level.**

that the fortified beverage powder received a greater number of oxidized flavor criticisms at most temperatures and storage times than the unfortified powder. The results are shown in Table 6.

TABLE 6

Variation with storage time of flavor intensity values for oxidized flavor for whey-soy beverage powder stored at three different temperatures

Storage temperature (°C)	Storage time - weeks								
	0	1	2	4	8	12	18	20	24
					Fortified				
–18.		.86	.29	.83	1.3	.67	.14	.67	.50
25.	1.0	.86	.29	.50	.50	.67	.29	1.5	.83
43.		.14	.29	.67	1.0	1.3	1.57	1.3	3.0
					Unfortified				
–18.		0	.14	0	0	0	0	.33	0
25.	0	0	.29	.16	.18	0	.14	.17	0
43.		0	0	.33	.83	1.0	.57	1.2	2.67

TABLE 7

Variation with storage time of peroxide values of whey-soy beverage powder stored at three different temperatures

Storage temperature (°C)	Storage time - weeks								
	0	1	2	4	8	12	18	20	24
					meq oxygen/kg fat				
					Fortified				
–18.		4.5	2.0	5.5	10.9	–	1.4	2.2	4.8
25.	7.7	4.7	10.2	12.6	19.4	–	30.5	15.1	8.7
43.		3.6	0.4	9.9	9.6	–	59.5	85.4	86.0
					Unfortified				
–18.		5.0	20.3	15.2	14.9	–	18.1	12.1	15.1
25.	10.3	5.9	19.4	21.9	23.3	–	40.9	38.2	44.3
43.		4.2	21.9	18.5	17.2	–	87.0	114	163

Analysis of variance showed the hedonic scores of the fortified samples during the first half of the storage study to be significantly lower than the scores of the unfortified sample set. Experienced judges could detect a flavor difference between the fortified and unfortified samples with regard to oxidized flavor, even though this difference was not reflected in the hedonic ratings during the latter half of the storage study. Rancid flavor criticisms were only made for those samples stored at 43° C whose hedonic flavor scores were significantly lower than controls.

Peroxide values for the samples stored at different temperatures for various times are shown in Table 7. Examination of the data show that those samples stored

at 43° C which had significantly lower flavor scores, had elevated peroxide values in most cases. The peroxide values for all samples of the fortified beverage powder were consistently lower at all storage times and temperatures than the peroxide values of the unfortified counterpart.

The presence of iron in the vitamin-mineral premix used to fortify the whey-soy beverage powder may offer an explanation for the oxidized flavor criticisms we observed in the early stages of the storage test as well as the decreased peroxide values of the fortified samples. List *et al.* (1974) have shown that an increase in iron content of soybean oil results in an increase in anisidine value, a measure of α,β unsaturated aldehydes contained in the fat. These compounds are formed by the breakdown of fat hydroperoxides; List *et al.* (1974) believe that iron may catalyze the formation of these products from the hydroperoxides and have correlated elevated anisidine values with decreased flavor score. Our results suggest that a similar effect may be occurring in the whey-soy beverage powder fortified with iron as one of the components of the vitamin-mineral premix.

CONCLUSIONS

The data presented here indicate that the new whey-soy beverage powder has the nutritional quality and storage stability required for use in international food donation programs. Acceptability tests carried out in six test countries by two consultants to USDA (Rodier *et al.*, 1973) using the fortified industry prototype, showed that there is a high probability that the beverage powder will be accepted in preschool feeding programs in most of the developing countries. The cost to produce the whey-soy beverage powder is estimated at 42.77ç/kilo (Moede, 1974), less than 15ç/liter of reconstituted beverage. The volume of nonfat dry milk shipments abroad under the Food-For-Peace Program was approximately 136 million kilos annually. It is estimated that annual shipments of the whey-soy beverage mix may reach a level of 55 million kilos in 2-3 years. As a food supplement for preschool children, whey-soy drink can help supply the calories, protein, and other nutrients necessary to meet the requirements of feeding programs in less-developed countries.

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Preparation and properties of protein concentrates obtained by wet-processing of cereal grain milling byproducts

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ABSTRACT

Protein concentrates were prepared from the byproducts resulting from the preparation of flour from cereal grains wheat, rice, rye, and triticale. The byproducts were extracted with dilute alkali. The extract was separated from the fibrous material and then treated with acid and/or heat to precipitate the protein. The protein concentrates prepared without removal of the starch fraction contained from 22-57 % protein, 5-50 % starch, and 7-30 % fat < 1 % fiber, and were obtained in yields of 15-25 %. When starch was removed from the alkaline extract before precipitation of the protein, products containing up to 80 % protein were produced. Nutritional studies, and functional properties relevant to food applications, are discussed.

INTRODUCTION

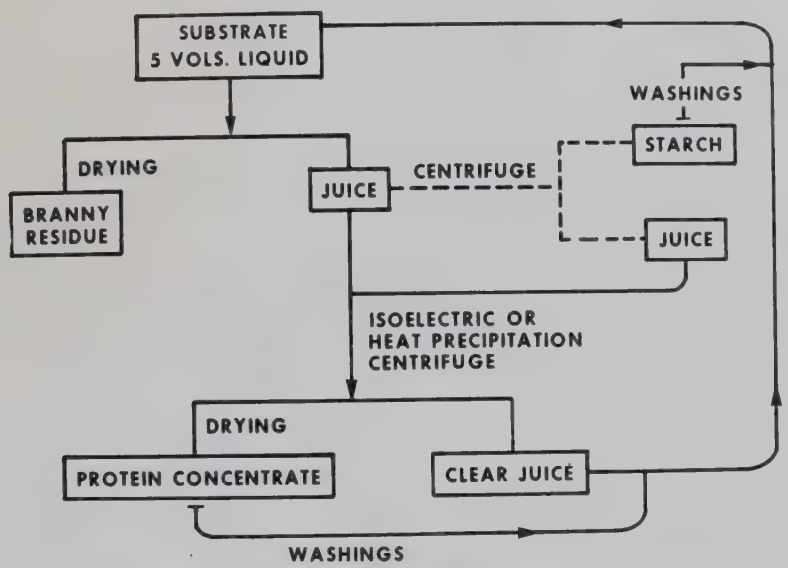
There are several reports in the literature on the preparation of protein concentrates from cereals by wet-processing. Reiners et al. (1972, 1973a, b) described the preparation of products with high protein content from corn gluten meal, corn, and sorghum. Products containing up to 89 % protein were prepared from oat millfeeds by alkaline extraction (Cluskey et al., 1973). A similar alkaline extraction process produced a concentrate containing 73 % protein from corn germ meal (Nielsen et al., 1973). Alkaline extraction of wheat bran or shorts (Fellers et al., 1966) and rice bran (Chen and Houston, 1970) yielded products high in protein and fat, but low in fiber.

Cereal grain milling byproducts are presently being inefficiently used as a ruminant feed. The protein content of these byproducts is high and the nutritional value is superior to flour protein. Their potential as a source of protein concentrates is obvious. Wheat milling byproducts in the United States alone represent about 800.000 tons of protein.

The objective of our work has been to concentrate the nutritious components of grain milling byproducts into products which could be utilized by nonruminants or humans, and use the remaining residue as a ruminant feed. We have accomplished this objective by using a wet alkaline process. This paper summarizes some of the work as carried out on milling byproducts of wheat rice, rye, and triticale. More detailed descriptions of the work relating to wheat (Saunders et al., 1975), rice (Connor et al., 1976), and triticale (Saunders et al., 1974) may be found in the literature.

EXPERIMENTAL

A flow-sheet describing the basic process is shown in Figure 1. The cereal grain byproduct is mixed with five volumes of water, adjusted to an alkaline pH with dilute sodium or ammonium hydroxide, and mixed for 15 minutes. The mixture is then pressed to separate the alkaline extract. The extract is subjected to either of two different processes. In one process, the extract is adjusted to pH 6.0, steam is injected to attain a temperature of 85° C, and the precipitated product is isolated by centrifugation. Alternatively, the protein is precipitated by acid (pH 4-5) and collected by centrifugation. In the second process, the alkaline extract is initially centrifuged to remove starch as a separate product, and the supernatant is then treated as in the first process. In both procedures the final supernatant ("clear juice" in Figure 1) plus any washes are recycled to the next batch of substrate and the process is repeated.



wet-processing of grain milling byproduct continuous batch extraction process

Fig. 1.–Flow-sheet showing preparation of protein concentrates from cereal grain milling byproducts.

RESULTS AND DISCUSSION

The influence of extraction time and temperature upon the yield of protein obtained from wheat, rice, and triticale brans at pH 8.6-9.0 was studied. At room temperature as well as 50-70° C, minimal differences were observed when the mixing time was increased from 15 to 60 minutes. From an economic viewpoint, it appears that at pH 8.6-9.0 an extraction time of 15 minutes at room temperature is adequate. (With the exception of data in Table 1, most of our in depth studies have been carried out at pH 8.6-9.0 (Saunders et al., 1974, 1975; Connor et al., 1976)

The yield and composition of some representative protein concentrates obtained from different cereal grain byproducts by alkaline extraction are listed in

TABLE 1

Yield and composition of protein concentrates from cereal grain milling by-products by wet-processing at different pH[1]

Substrate	pH	Yield %	Protein %	Fat %	Fiber %	Ash %	Starch %
Wheat shorts.	8	20.0	35.2	6.9	0.4	5.1	–
	9	22.3	35.1	11.1	0.4	3.9	36.5
	10	25.1	40.7	10.9	0.3	4.1	38.4
Wheat millrun.	8	18.4	27.2	7	0.8	4.5	49.3
	9	20.1	26.2	7.0	0.8	4.1	47.7
Wheat bran.	9	12.6	34.7	9.6	0.8	6.6	40.6
Rice bran.	8	22.1	21.8	30.8	0.8	12.9	–
	9	21.5	22.7	32.7	0.7	11.7	22.9
	10	22.7	27.7	32.0	0.7	10.9	22.4
Rye middlings.	8	23.3	30.5	1.9	0.3	5.1	45.8
	9	25.0	33.2	3.9	0.5	4.8	42.1
	10	27.4	32.7	3.6	0.4	3.1	38.6
Triticale Bran.	8	11.0	57.0	12.5	0.3	6.0	4.9
	9	16.2	47.7	11.8	0,3	5.3	16.7
	10	21.4	42.8	6.9	0.3	4.3	21.2

[1] Moisture-free basis, N X 6.25.

TABLE 2

Yield and composition of protein concentrates from grain milling by-products by wet-processing at pH 8.6-9.0. Starch removed during the process[1]

Substrate	Yield %	Protein %	Fat %	Fiber %	Ash %
Wheat shorts.	11.2	65.3	17.7	0.5	0.6
Wheat millrun.	7.6	64.1	19.2	0.4	4.7
Rice bran.	12.0	39.8	54.2	0.3	2.3
Triticale bran.	6.7	71.7	20.9	0.3	1.3

[1] Moisture-free basis, N X 6.25.

Table 1. The yield and composition of products where starch was removed as a separate product prior to protein precipitation are described in Table 2. Data for products obtained during a recycling operation in the cases of wheat shorts and rice bran are shown in Table 3. The compositional differences in the products within the recycling operation are minimal, and it is thus probably feasible to operate the process in this manner. Drying costs in a recycling operation would be considerably lower than that of a singlebatch operation.

The residue left after extraction of protein concentrates from each of the cereal grain milling byproducts discussed is similar in composition to that of the cereal bran. *In vitro* evaluation (Guggolz et al., 1971) of the residues indicated Total Digestible Nutrients (TDN) values which are acceptable for ruminant rations. (The liquid extractant ("clear juice," Fig. 1) was high in protein and sugars, (glucose,

TABLE 3

Yield and composition of protein concentrates obtained by alkaline extraction of wheat shorts and rice bran at pH 8.6-9.0, during a recycling procedure[1]

Recycle batch no.	Yield %	Protein %	Fat %	Fiber %	Ash %	Solids in recycle juice %
Wheat shorts						
1.	21.7	36.8	9.2	0.2	4.9	3.0
3.	20.4	33.0	6.3	0.3	5.4	5.7
5.	20.6	29.7	6.2	0.3	5.4	7.3
8.	21.2	32.7	5.4	0.4	6.2	7.3
Rice bran						
1.	16.9	26.9	36.6	12.6	12.6	2.9
3.	16.4	27.5	34.4	10.5	10.5	5.9
5.	15.5	28.8	34.2	10.7	10.7	6.3
10.	15.6	26.9	30.5	11.4	11.4	6.5

[1] Moisture-free basis, N X 6.25.

fructose, sucrose, raffinose, maltose, and neokestose) and after concentration should be suitable as a feed supplement or a growth medium for unicellular organisms.

Trace minerals and some vitamins were concentrated in the products. When compared to the respective cereal flours, the concentrates had substantially higher levels of iron, and vitamins B_1 and B_2.

The concentrates appear to be quite stable. After several months storage at room temperature there was no appreciable rancidity as found by odor. Storage studies on the wheat protein concentrates have been described in detail elsewhere (Betschart et al., 1975). The fatty acids of wheat protein concentrates were identified as mainly linoleic (54 %), oleic (23 %), palmitic (20 %), and linolenic (4 (4%), whereas, those of rice protein concentrates were oleic (49 %), linoleic (37 %), and palmitic (14 %), and those of triticale protein concentrates were linoleic (54 %), palmitic (28 %), oleic (17 %), and linolenic (6 %). The high concentration of unsaturated fatty acids is favorable from a human nutrition aspect.

In feeding studies with rats, Protein Efficiency Ratio (PER) and nitrogen digestibility were determined for concentrates from wheat, rice, and triticale milling byproducts (Table 4). The values for PER and nitrogen digestibility compare favorably with values in the literature for other plant protein concentrates (FAO, 1970). The method of drying did not affect the nutritive values measured.

The concentrates could be used effectively as feeds in poultry rations and in weaning rations of calves and pigs. Their theoretical metabolizable energy value ranges from 3500 to 4500 Kcal/Kg., which compares favorably with present commercial chick feeds. Actual values determined in our laboratory for concentrates from wheat shorts and millrun were 3652 and 3762 Kcal/Kg., respectively (Kuzmicky, 1974).

The potential for these concentrates in human foods in currently under investigation. Functional property parameters such as protein solubitliy profiles, water absorption, dispersibility, and baking quality, have been evaluated on some concentrates and will be published elsewhere (Betschart et al., 1974). By way of

TABLE 4

PER and nitrogen digestibility of protein concentrates from cereal grain milling by-products by wet-processing at pH 8.6-9.0

Material	PER[1]	Nitrogen digestibility[2]
Casein.	2.50	100
Wheat concentrate, acid ppt[3].	1.74	93.8
Wheat concentrate, heat ppt[3].	1.81	92.1
Wheat concentrate, heat ppt[4].	1.88	92.1
Wheat concentrate, heat ppt[5].	1.91	91.2
Rice concentrate, acid ppt[3].	1.99	89.6
Rice concentrate, heat ppt[3].	2.19	83.4
Triticale concentrate, acid ppt[3].	1.81	95.1

[1] Corrected to casein value of 2.50.
[2] Corrected for metabolic nitrogen excretion.
[3] Freeze-dried.
[4] Drum-dried.
[5] Spray-dried.

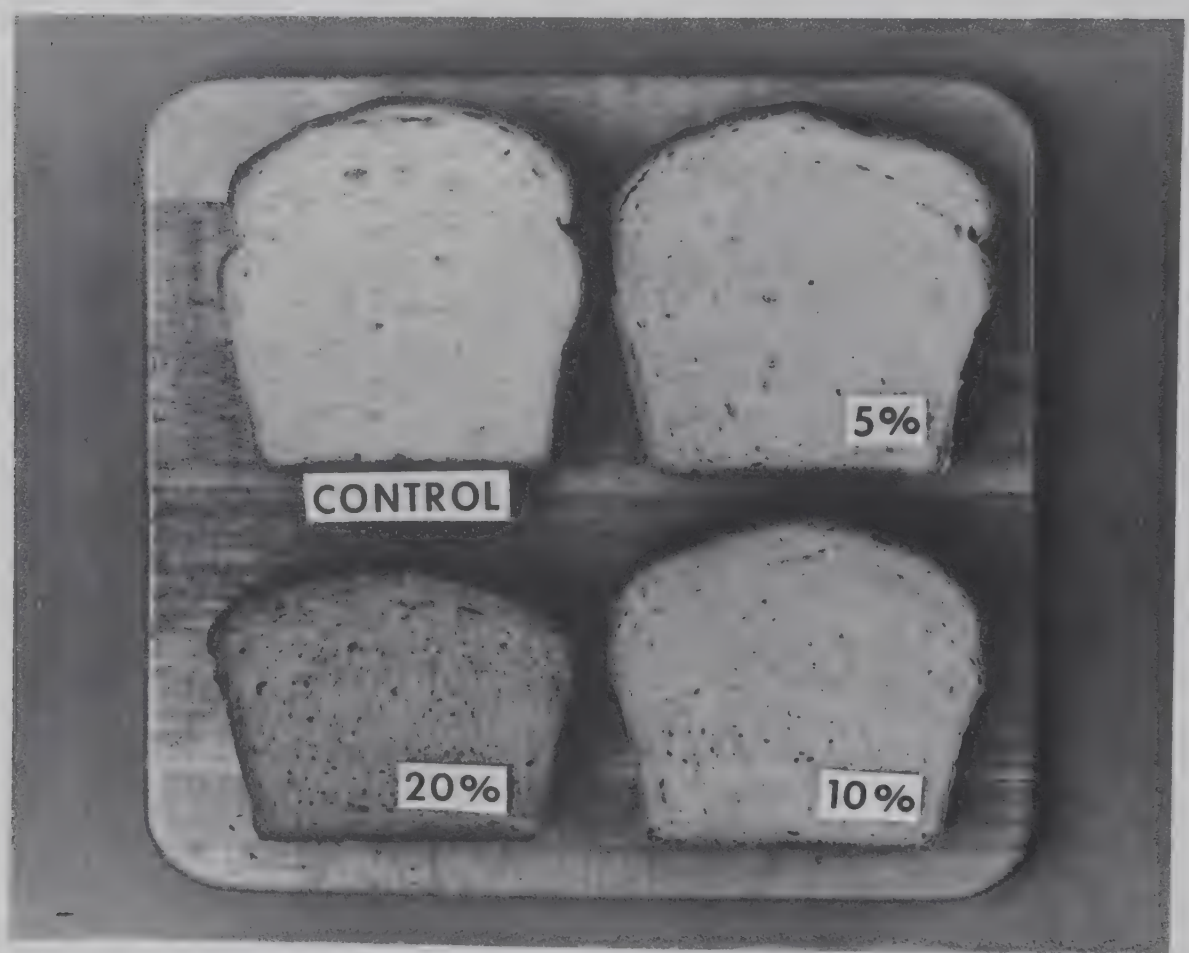


Fig. 2.–Fortification of wheat bread (control) with 5, 10, and 20 % protein concentrate prepared by alkaline extraction of wheat shorts.

example, Figure 2 shows the result of fortifying wheat bread with up to 20 % of a protein concentrate prepared from wheat millrum by the wet alkaline process. The protein content of the fortified breads shown here was increased by as much as 50 %, and the PER values of the fortified breads were nearly double that of the control wheat flour bread.

In conclusion, this brief report demonstrates that protein concentrates can be manufactured from various cereal grain milling byproducts. It remains to be seen however, if the process can be carried out commercially, and if the concentrates, in addition to nutritive value, prove to have properties desirable for human foods.

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Faba beans (*Vicia faba minor*) – physical, chemical and nutritional properties

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ABSTRACT

Ultrastructure studies with the ordinary light and transmission electron microscopes of the mature and developing bean have shown that the protein is deposited in the cotyledons in discrete protein bodies ranging in size from 1 to 5 microns. The microscopic studies have shown the presence of a membrane in the vacuoles that has not been observed previously. The total bean comprises about 15 % hull and 85 % cotyledon. The cotyledon contains 47 % starch, 34 % protein (N × 6.25), 1.3 % free lipid, 7.2 % soluble carbohydrate, 1.1 % crude fiber and 3.1 % ash. The starch comprises about 69 % amylopectin and 31 % amylose. Fababean starch gelatinizes at a lower temperature than wheat starch and sets to a slightly higher cold paste viscosity. Fababean protein is essentially all of the globulin type comprising two major proteins, legumin and vicilin. The amino acid composition of fababean protein is typical of legumes having a relatively high proportion of lysine and low proportion of methionine. Nutritional assessment with rats showed that fababean protein supplements with 0.125 % DL-methionine gives about the same weight gain as casein at the same protein level. High protein bread made from a composite wheat-fababean flour compared favourably in nutritional value (by rat assay) with similar bread made from wheat-soy composite flour.

INTRODUCTION

Pressures on the agricultural community to keep up, or in fact to catch up, with the world-wide demand for protein is well known. The future importance of legume proteins in meeting this demand is continually being expressed. Although plant protein production in Canada far exceeds the local needs, legume protein,

mainly in the form of soybean meal, is imported into Canada in large quantities each year. As a result of continuing investigations into locally grown crops to satisfy this market, as well as the export market, considerable interest has developed in fababeans (*Vicia faba* L. minor) as a potential high protein crop.

Although fababeans have been grown extensively in many areas in Europe, Asia and Africa since the beginning of recorded history, various agronomic deficiencies have resulted in limited acreage in the 20 th century. Recent development of more desirable varieties, mainly in Germany, is the major reason for regenerated interest in fababeans. Preliminary reports on production yields and feeding trials for these newer varieties grown in Canada indicate that fababeans may be a valuable feed for livestock and poultry.

The use of fababeans as a food or as an ingredient in food products is also receiving additional attention. Ranks Hovis McDougall Ltd. in England has produced protein isolates from fababeans which can be spun into simulated meat fibers. Workers at Kansas State University (Patel and Johnson, 1972) and at the University of Manitoba (McConnell *et al*, 1974) have shown that fababean flour and protein isolates can partially replace wheat flour in bread. Much additional information concerning the chemical and physical properties of fababeans and the seed components is required, however, if additional food applications are to be successful.

The object of this research was to provide information on the physicochemical properties of the seed components of Canadian grown fababeans.

MATERIALS

Fababean seeds from the cultivar Ackerperle were obtained from plants grown on experimental plots at the University of Manitoba in the 1973 growing season. The seed was stored at 6-8° C prior to dehulling and grinding. Flour samples were stored in sealed metal containers prior to testing.

EXPERIMENTAL

Compositional Studies

Analyses for protein, lipid, ash, and crude fiber were performed using the American Association of Cereal Chemists (A. A. C. C.) standard methods. Starch was determined using the glycoamylase method presently under investigation by the Starch and Pentosan Collaborative Group of the A. A. C. C. Water-soluble carbohydrates were determined by testing for total carbohydrate in the filtrate of a water extract of fababean flour (1 : 12.5 flour to water ratio).

Microscopic Studies

Mature and developing seeds were prepared for microscopic examination under the light microscope according to the method of Feder and O'Brien (1968). Samples were carefully sliced approximately 1 mm. thick and fixed in 3 % glutaraldehyde, dehydrated and embedded in a glycol-methacrylate monomer mixture. Microtome sections of 2 to 3 microns thick were stained with toluidene blue or fast green and photographed through a Zeiss Photomicroscope II using Kodak ASA 30 black and white film.

Transmission electron microscopic investigations of fababeans were made on seed cotyledons at various stages of development. Fresh seeds were sliced approximately 1 mm. thick and fixed overnight at 4° C in 6 % glutaraldehyde solution in 0.025 M. phosphate buffer (pH 6.8). After washing with buffer, the samples were fixed for 15 hours in 1 % osmium tetroxide, then washed again in phosphate buffer and rinsed with distilled water. Samples were dehydrated by equilibrating in a series of ethanol solutions of increasing concentrations followed by dry acetone and finally infiltrated with Spurr's (1969) low viscosity epoxy resin.

A second series of samples was prepared similarly using cacodylate instead of phosphate buffer.

Th 6 % glutaraldehyde solution was prepared in 0.2 M cacodylate buffer (pH 7.35) and a 0.1 M buffer concentration was used in the washing steps. Samples were stained overnight in 0.5 % uranyl acetate, prepared in 0.1 M buffer, prior to dehydration.

Epoxy sections showing grey to silver interference colors were placed on 300 mesh coated copper grids and stained for 10 to 15 minutes with lead citrate (Reynolds, 1963). Samples prepared in phosphate buffer were also stained for 10 to 15 minutes in 0.5 % uranyl acetate solution.

Preparations were examined on an AEI Modelo 6B transmission electron microscope and photographed using Kodak electron sensitive emulsion on glass plates.

Starch Pasting Curves

Starch pasting curves (amylograms) were obtained with a Brabender VISCO/AMYLO/GRAPH according to the A. A. C. C. Approved Method 22-10. Fababean starch was prepared by extracting fababean flour with 0.016N sodium hydroxide to remove the solubles, followed by washing the crude starch three times with water, twice with 70 % ethanol and two additional times with water. After freeze drying, the starch preparation was ground to pass through a 9XX screen.

Pasting curves were obtained using sample weights of 65 g (ambient moisture-approximately 10 %) for the flours and 36 g (dry weight basis) for the starches. All samples were dispersed in 450 ml of tap water and adjusted to 30° C. With the bowl speed set at 78 r. p. m. the temperature was increased at a rate of 1.5° per min. to 95°, held at this temperature for 20 minutes, then decreased at 1.5° per min to 35° and held at this temperature for 20 minutes.

Air Classification

Fababean flour containing 33.6 % protein was reduced in particle size by a single pass through an Alpine 160Z pin mill operating at 16.500 r. p. m. The flour was then separated into a protein-rich fraction and a starch-rich fraction using an Alpine Mikroplex Model MP Air Classifier.

Protein Solubility

Fababean protein solubility was measured over a wide range of pH levels as described by Smith and Circle (1938).

Protein Isolate Production

Fababean flour was stirred for 2 hours in dilute (0.016N) sodium hydroxide solution 1 to 10 (w/v) at pH 8.5. The soluble protein was separated from the residue by centrifugation for 20 minutes at 3.000 x g. The residue was washed once with water and the supernatants were combined. The majority of the protein was then recovered from the supernatant by slowly adjusting the pH to 4.0 with 1N hydrochloric acid and separating the proteinaceous precipitate by centrifugation. This protein isolate was washed once with water and freeze-dried.

The insoluble residue and the whey solution were also quantitatively recovered and freeze-dried. The dry weights as well as the protein contents of each fraction were determined and recorded.

Amino Acid Analyses

Amino acid compositions were determined on a Beckman Model 121 automatic amino acid analyzer using the standard 4 hour procedure. Concentrations of individual amino acids were computed against a Beckman standard. The proteins were hydrolyzed under vacuum with 6N hydrochloric acid and a hydrolysis time of 24 hours at 110° C.

TABLE 1

Data for hand separation of fababean seeds showing average seed weights[1] and percentages of cotyledon and hull

Variety	Seed wt. (g)	Cotyledon (%)	Hull (%)
Ackeperle.	0.30	84.8	15.2
Diana.	0.43	87.4	12.6
Erfordia.	0.49	87.1	12.9

[1] On an 'as is' moisture basis.

Nutritive Value Studies

Ground dehulled fababean (31 % crude protein, N x 6.25) was fed ro rats over a 21-day growth trial. The daily gain and the daily food intake for fababean flour plus three levels of DL-methionine were measured using casein as a standard.

Growing rats (10 per treatment group) were fed isonitrogenous diets (10 % protein) in which the protein was supplied by : 1) wheat flour, 2) fababean protein enriched bread, or 3) soybean protein enriched wheat flour bread. Bread supplied the only source of protein in each diet during the 21-day experimental period.

RESULTS AND DISCUSSION

Compositional Studies

Data showing average seed weights and percentages of cotyledon and hull obtained by hand separation are shown in Table 1. An inverse relationship between seed size and percentage of hull was expected. Although soybean seeds are approximately one half the size of fababean seeds, the hull content accounts for only about 8 % of the soybean seed weight. Thus on considering the differences in percent hull and seed size, the greater thickness of the fababean hull compared with soybean hull can be appreciated.

The compositional analysis data for the fababean sample used in the present study is presented in Table 2. The levels of protein, lipid, ash starch fiber all agree with published values for European varieties (Eden, 1968). Analysis of a number of replicates (N = 14) of hull-free fababean flour gave a mean protein level of 33.8 % with a standard deviation of 0.4. Subsequent analysis of a second variety of fababean (i. e. Diana) indicated protein levels of 31.7 % for the whole seed and 37.3 % for the dehulled seed. The starch values in Table 2 represent an average of 6 determinations. Values ranged from 46.1 % to 47.4 % which agrees with values reported by Cerning *et al* (1973). The amount of carbohydrate found in the water extract of fababean flour was determined in order to determine the amount of carbohydrate which was solubilized in the protein fractionation procedure.

TABLE 2

Composition of fababean seeds (%, dry basis)

	Seed %	Dehulled Seed %	Hulls %
Protein	29.8	33.9	5.6
Lipid	1.2	1.3	0.2
Ash	3.0	3.1	2.4
Starch	–	46.8	–
WS Carbohydrate	–	7.2	–
Crude Fiber	7.8	1.1	45.8

Light Microscopy

Light micrographs showing the structure of fababean cotyledon cells at various stages of development are shown in Figs. 1, 2 and 3. As the primary concern of this investigation was to follow the formation of the protein bodies, the protein specific stain, fast green, was used for staining sections prepared from seeds which were at the early stages of storage protein deposition. Sections prepared from seeds

at earlier and later stages of development were stained with toluidene blue due to its overall superiority to fast green for showing the total cellular structure.

Preliminary investigations had indicated that protein bodies began to appear between 20 and 30 days after anthesis.

Figure la shows a cross-section of a seed 10 days after anthesis. At this stage, meristematic cells (M) can be seen at the surface of the seed. However, inner cotyledon cells (Cot) have already begun to undergo vacuolation. The average cell diameter at this stage of cotyledon development was about 25 microns, with the nucleus measuring about 15 microns in diameter.

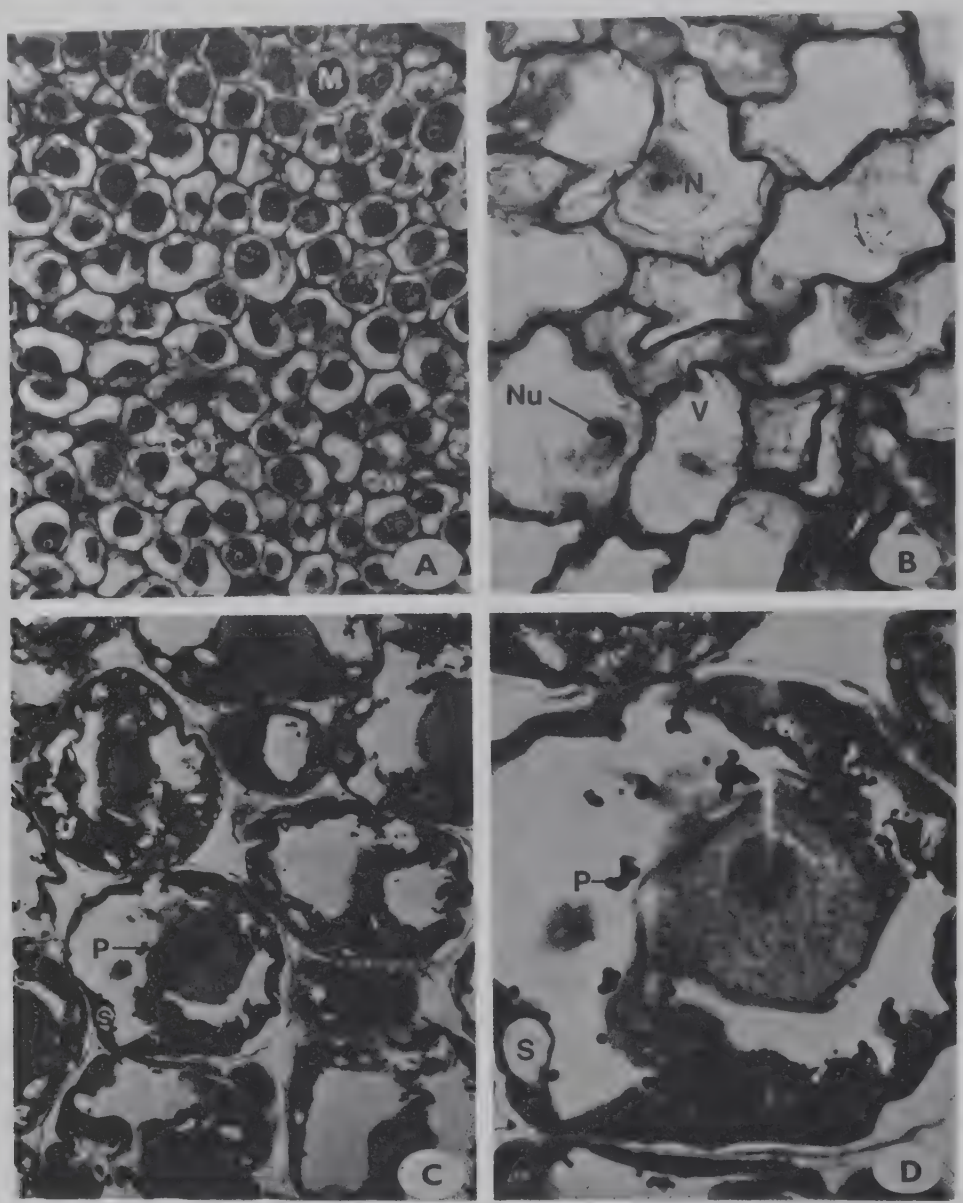


Fig. 1.–Light micrographs of developing fababean cotyledon cells showing: A. 10 days from anthesis (602X); B. 20 days from anthesis (2,410X); C. 30 days from anthesis (2, 410X) and D. 30 days from anthesis (6, 020X).
Legend: Meristematic cells (M), cotyledon cells (Cot), nucleus (N), nucleolus (Nu), vacuoles (V), protein (P), starch (S).

By 20 days after anthesis (Fig. 1b), the cotyledon cells had increased considerably in size and had become highly irregular in shape. Vacuolation had advanced to the point where the nucleus and the cytoplasm became restricted to the periphery of the cell and occupied less than a quarter of the volume of the cell. Neither starch granules nor protein bodies could be seen at this stage of development.

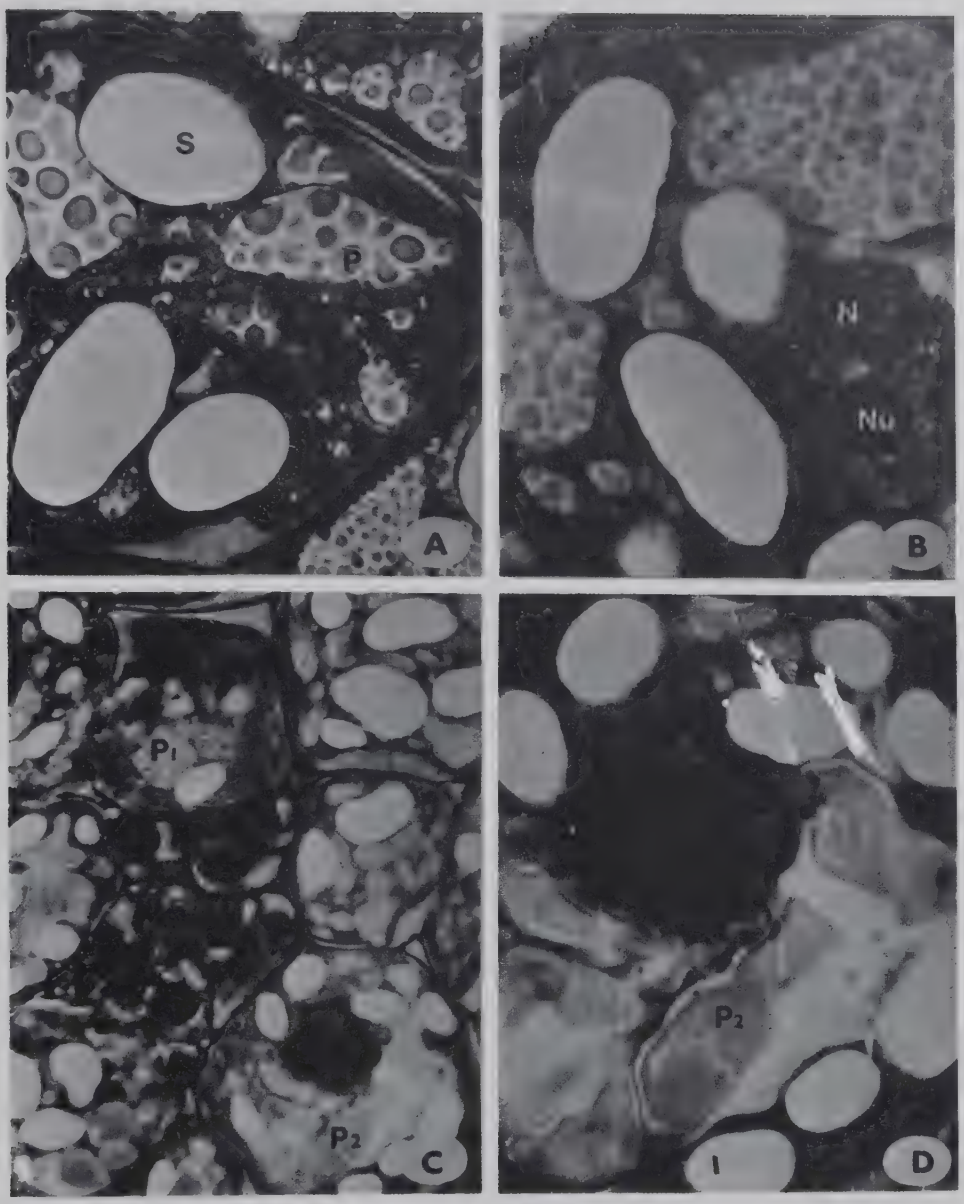


Fig. 2.—Light micrographs of fababean cotyledon cells 35 days from anthesis showing: A and B. Protein bodies within the vacuoles (P), starch (S), nucleus (N), and nucleolus (Nu), (6,020X); C. Type 1 protein bodies (P_1), type 2 protein bodies (P_2), and a vascular bundle (VB), (2,410X); D. Type 2 protein bodies (P_2), (6,020X).

Figures 1c and 1d illustrate typical cotyledon cells at 30 days after anthesis. The small heavily stained bodies seen mainly around the periphery of the vacuoles are the early forms of storage protein. Many of these protein masses are seen as individual spheres, but aggregates of these spheres are also quite common. The individual protein spheres ranged in size up to approximately 1 micron in diameter. Some small protein bodies also appeared to be forming within the cytoplasm, but greater resolution is required to determine if these bodies were actually forming in the cytoplasm or in smaller vacuoles. Also seen in the cytoplasm are small starch granules ranging up to about 8 microns in diameter.

Cotyledon cells at 35 days after anthesis are shown in Fig. 2. The toluidene blue used to stain these sections gave a medium intensity of color to the storage protein. The starch granules were completely free of stain while the nucleus and nucleolus stained to a dark color.

The rapid increase in protein and starch content during the 30 - 35 day stage is readily observed. By 35 days, the starch granuls are almost full size measuring

about 20 - 30 microns along the long axis of the granule, and have assumed the typical oblong or lobed shapes observed by scanning electron microscopy. The protein spheres have increased in size to the 1 micron to 5 micron range observed in the mature seed. The large vacuoles have either become highly irregular in shape or sub-divided by strands or sheets of cytoplasm. Few vacuolar areas measured more than 50 microns in length at this stage of development (see Figs. 2a and 2b).

On examining cross sections of a number of cells at the 35-day stage of development, two types of protein deposits were observed. About 70 % to 80 % of the cells contained small protein spheres of 1 micron to 5 microns in diameter, most of which were within the larger vacuoles. This type of protein accumulation (hereafter designated as type 1 protein body formation, or type 1 cells) is seen in Figs. 2a and 2b. A second type of protein accumulation (type 2) was observed in which large amorphous masses of protein were found within the vacuoles. Type 2 protein body formation is shown in several cells in the lower portion of Fig. 2c. A higher magnification of one of these cells is shown in Fig. 2d.

The type of protein body formation within a single cell appears to be the same

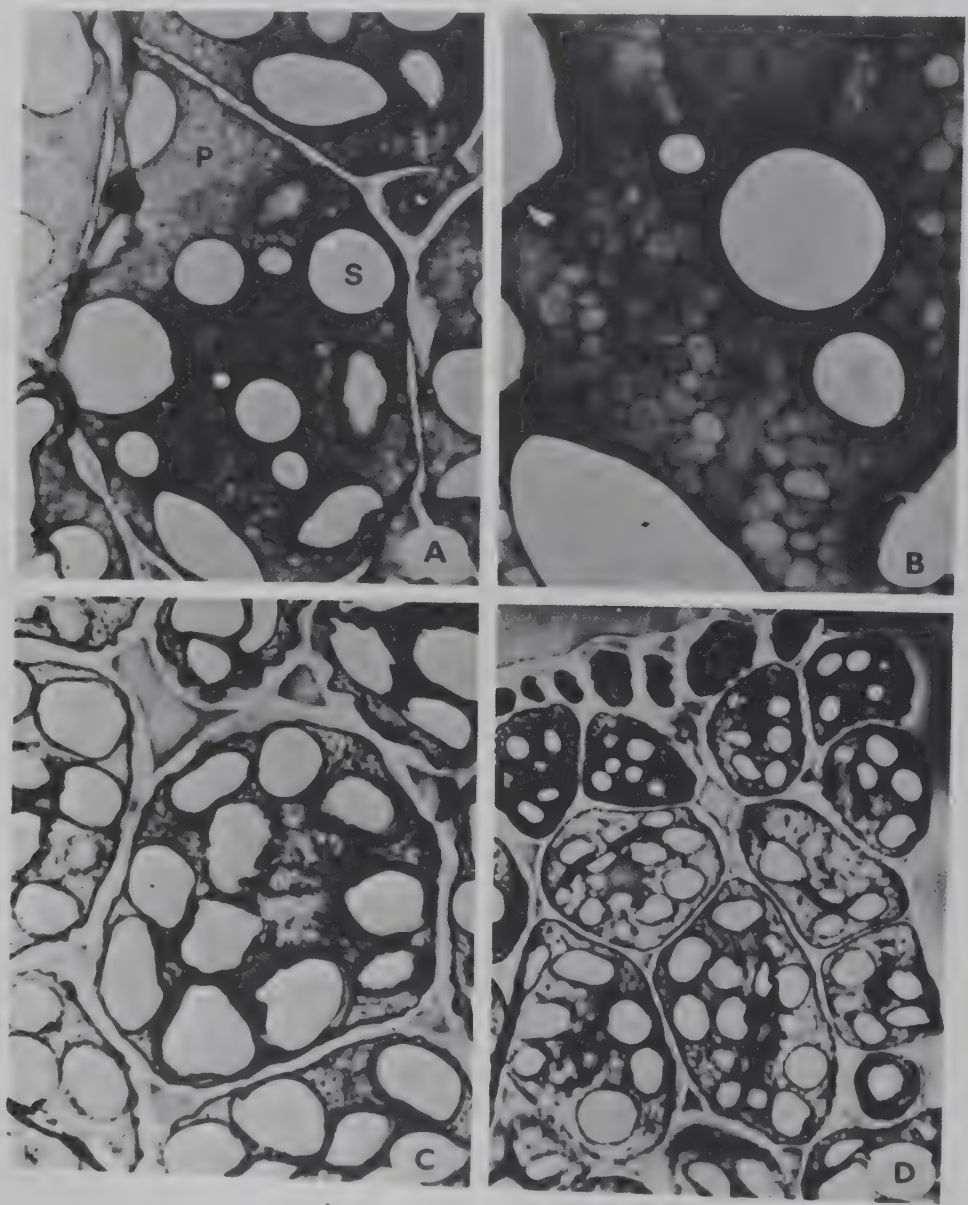


Fig. 3.—Light micrographs of mature fababean cotyledon cells showing protein (P) and starch (S). A. (2,410X); B. (6,020X); C. and D. (2,410X).

throughout a cell although adjacent cells can exhibit different types of protein body formation (see Fig. 2c). It was generally observed that type 2 cells were more abundant near the surface of the cotyledon or adjacent to vascular bundles, but several exceptions were noted to this observation. A few vascular cells can be seen near the center of Fig. 2c.

At maturity (60 to 70 days after anthesis) little could be seen in the cells other than starch granules, protein bodies and the nucleus (Fig. 3). Although areas of different staining intensity were noticed in the cells, no distinction could be seen between the vacuolar areas and the cytoplasmic aresas when viewed through the light microscope. Some differences in staining intensities among individual protein bodies were noticed. Further testing would be required to determine if the differences were due to actual differences in the protein or to anomalies in the staining procedure.

As a result of starch and protein deposition and the dehydration during the latter stages of seed development, the protein bodies become tightly packed throughout the cell. This causes the protein bodies to become polyhedral (Fig. 3b) or whirled (Figs. 3c and 3d) in shape. The whirled arrangement of the protein bodies may indicate the that the starch granules are still growing after protein deposition has finished, or that the cells undergo distoriton during the dehydration process.

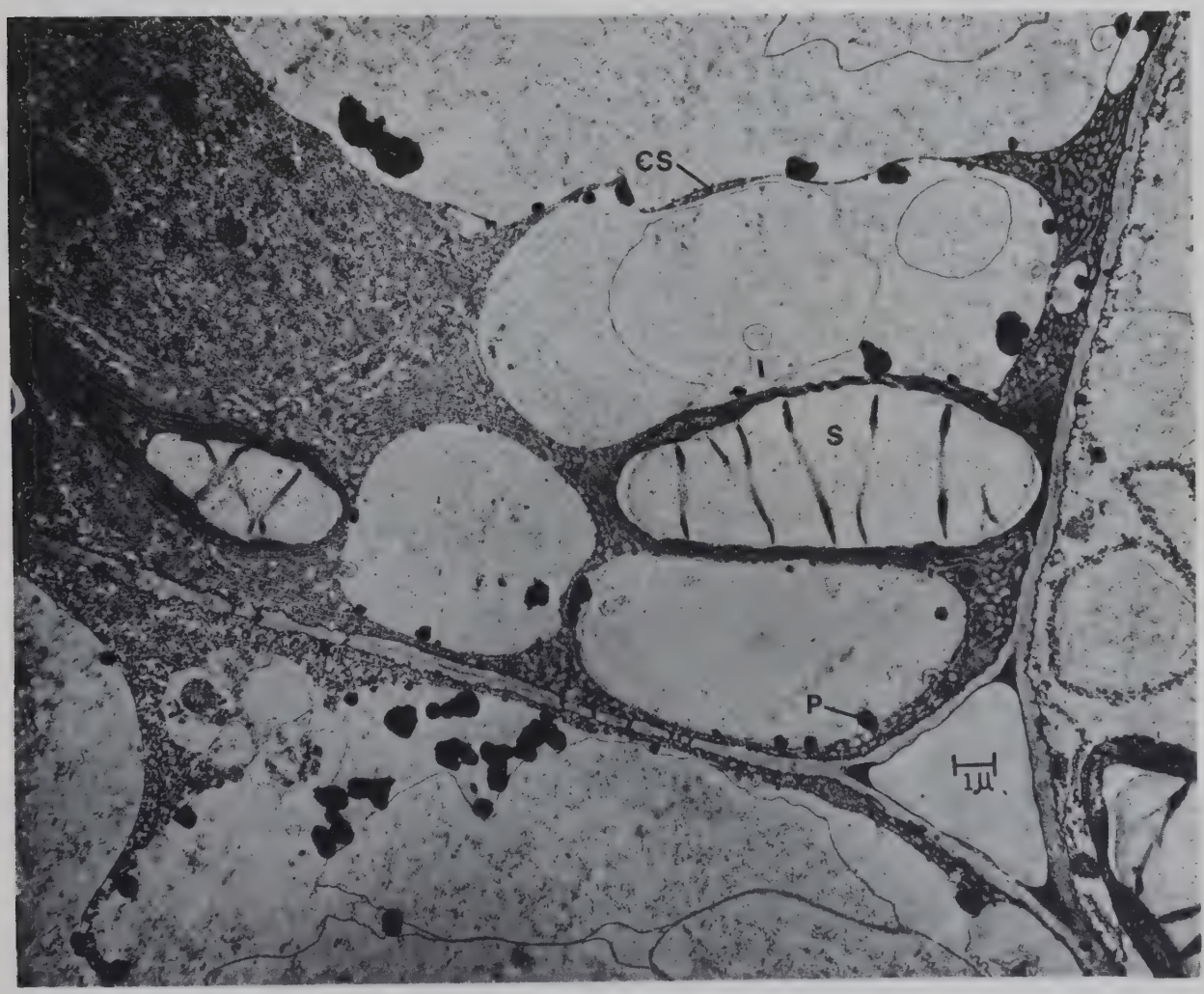


Fig. 4.–Transmission electron micrograph of fababean cotyledon cells at an early stage of storage protein deposition, showing: storage protein (P), starch (S), and cytoplasmic strands (CS).

Transmission Electron Microscopy

In attempts to further ellucidate some of the findings of the light microscope study, photomicrographs were taken of fababean cotyledon cells at various stages of development using a transmission electron microscope (TEM).

The initiation of storage protein synthesis in cotyledon cells was marked by the appearance of darkly stained masses around the perphery of the vacuoles (Fig. 4). This stage of development corresponds to approximately 35 days after anthesis (refer to Fig. 1). The proliferation of membrane within the vacuoles was readily apparent at this stage. Some of the large vacuoles appeared to be sectioned by strands or sheets of cytoplasm. It is possible, however, that these vacuoles may still be continuous with one another in the three dimensional setting of the cell.

At this stage, the starch granules have begun to push the contents of the chloroplasts to a thin layer around the periphery of granules.

Considerable variability was observed in the nature of the cytoplasm during the early stages of storage protein deposition. Some areas appeared to stain fairly heavily while others appeared to have only a few strands of rough endoplasmic reticulum (RER) scattered throughout large areas. Whether this variability exists **in** *vivo* or whether it was produce by the disruption of the cell during preparation is not known.

Accumulating storage protein is shown at still higher magnification in Fig. 5. At this early stage of storage protein deposition, individual protein synthesizing systems appear to give rise to individual accretions of protein around the periphery of the vacuoles.



Fig. 5. –Transmission electron micrograph showing: developing protein bodies (P), the tonoplast membrane (TM), vacuolar membrane (VM), lumen of the endoplasmic reticulum (L), ribosomes (R), and middle lamella (ML).

The roughly spherical areas of low stain intensity observed in the cytoplasm in Fig. 5 are believed to be the lumen of the RER. The area surrounding these tubules contains cytoplasm which is densely populated with ribsosomes. The generally accepted hypothesis concerning the transport function of the endoplasmic reticulum (ER) could not be confirmed in this study as no direct connection could be found between the ER and the developing storage protein masses. On the contrary, the protein seemed to accumulate opposite the cytoplasmic areas and not the ER tubules.

Where two or more sites of storage protein deposition were in close proximity, the developing protein masses fused into larger irregularly shaped protein accretions (see Fig. 4). It is possible that the vacuolar membrane may prevent the fusing of individual accretions of protein in cases where the membrane surrounds the developing protein bodies (see Fig. 5).

As the seed matures and becomes very dry specimen preparation was more difficult. Dehydration and subsequent infiltration with the viscous Spurs resin was greatly impeded, and the preparation of thin sections with glass knives was most difficult.

The general appearance of the mature seed in which the starch granule and the protein bodies constitute most of the cell contents, can be seen in Fig. 6. The cell walls are still prominent and have become thickned at the corners of the cells.

Variability in staining intensities of the protein bodies was evident. The protein bodies observed in Fig. 6 range in size from about 1 micron to 3 microns. These are probably the "type 1" bodies previously described. The small unstained

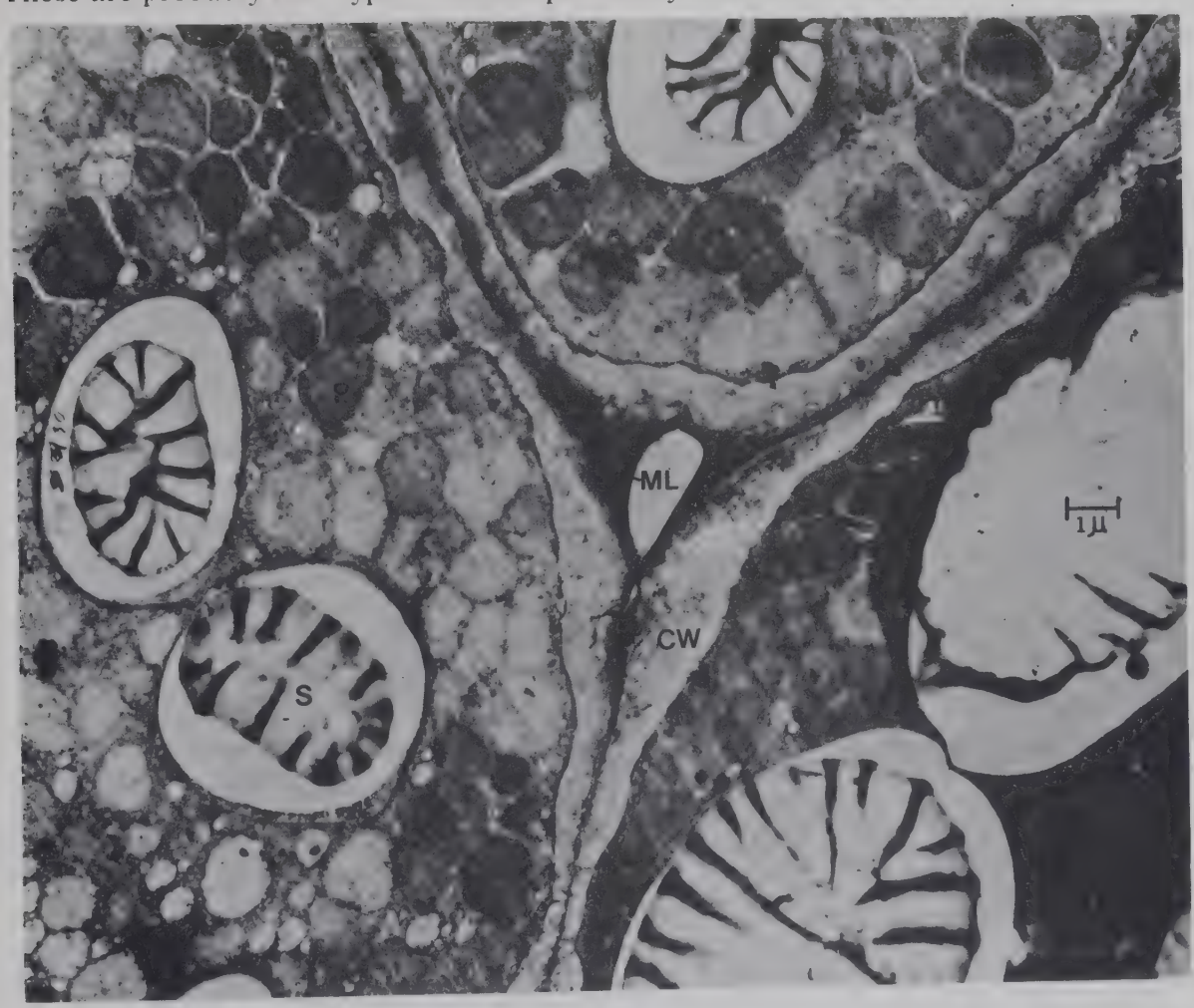


Fig. 6.–Transmission electron micrograph of mature fababean cotyledon cells, showing: cell walls (CW), middle lamella (ML), protein bodies (P), and starch (S).

spheres observed in Fig. 6 are presumed to be artifacts caused by trapped air or water in the specimen.

Pasting Curves

Figure 7 compares the pasting curves obtained for fababean and wheat products. The top set of curves is for a crude starch preparation from fababeans and a commercial samples of wheat starch. The bottom set compares fababean flour with wheat flour milled from Manitou wheat of 13.2 % protein content.

Fababean starch shows greater viscosity during the initial temperature rise, continued thickening through the hold at 95° and a higher cold paste viscosity than the wheat starch.

The two curves for the flours were quite similar with the exception of the higher peak in viscosity shown by wheat flour at the beginning of the 95° hold. A greater rate of thickening was also observed for wheat during the hold period at 35°. Since the same weight of flour was used to obtain both curves, the amount of starch in the wheat sample was almost double that in the fababean sample. Thus, the superior thickening ability of fababean starch as compared with wheat starch is shown in both the starch and flour pasting curves.

Amperometric titration of the fababean starch indicates an amylose content of 30.7 %.

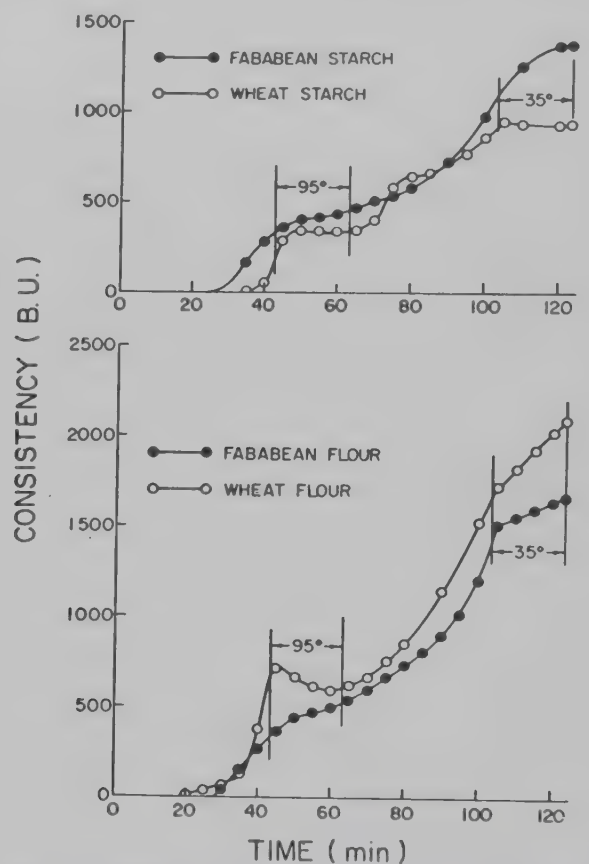


Fig. 7.–Pasting curves for fababean (var. Ackerperle) and wheat (var. Manitou). (Upper curves, starches = 40 g in 450 ml water; lower curves, flours - 65 g in 450 ml water).

Air Classification of Fababean Flour

The shift in protein content resulting from air classification of pin milled fababean flour is shown in Table 3. These data indicate that a protein rich flour containing about two times the initial protein content can be obtained. Through the use of this simple technique 87 % of the protein can be recovered in this fraction.

TABLE 3

Air classification of fababean flour[1]

	Amount %	Protein %
Protein-rich fraction.	45.9[2]	63.8
Starch-rich fraction.	54.1	5.8

[1] Protein content of original flour = 33.6 % (N × 6.25).
[2] Protein recovery in protein-rich fraction = 87 %.

Protein Solubility

The percentages of fababean flour nitrogen which were solubilized over a pH range from 1.4 to 10.6 are shown in Fig. 8. Solutions of varying concentrations of hydrochloric acid and sodium hydroxide were used to obtain the desired pH. Acid concentrations ranged from zero at pH 6.5 up to 0.125N at pH 1.5. A concentration of 0.04N was required to produce minimum protein solubility. Base concentrations ranged from zero at pH 6.5 to 0.07N at pH 11.3. Also included in Fig. 8 for comparison is a curve representing the solubility of soybean flour nitrogen as determined by Smith and Circle (1938). Several similarities were found on comparing these two curves. First, the isoelectric pH of about 3.9 reported here for fababean proteins is very close to the pH value of 4.1 generally reported for the isoelectric point of soybean proteins. Second, the maximum nitrogen solubility for both species is found in the alkaline region. Third, a steep part of both curves is found at approximately pH 6.0. And fourth, the difference in percent soluble nitrogen between the mild alkaline region and the isoelectric point is approximately the same for both species. The major differences in the two curves include the wider isoelectric range for the fababean protein, and the lower solubility of the fababean nitrogen compounds in the pH 2 to 3 region.

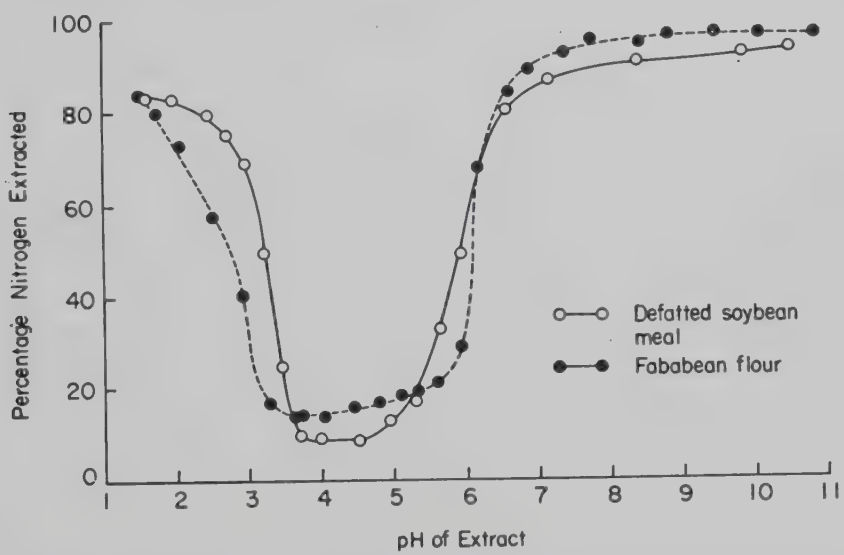


Fig. 8.–Extractability of proteins in fababean flour (var. Ackerperle) and defatted soybean meal as a function of pH. (Soybean values taken from Smith and Circle, 1938).

Protein Isolate Production

Results of experiments concerning the distribution and recovery of protein fractionated from fababean flour using pH adjustment techniques are shown in

TABLE 4

Percentage recovery by weight[1] of fractions obtained from the protein isolation procedure

Fraction		Recovery (%)
Isolate.	(88-90 % protein)	28-30
Residue.	(4- 6 % protein)	54-56
Soluble fraction.	(32-35 % protein)	14-16

[1] On a dry weight basis.

Table 4. The 28 % to 30 % of the initial seed weight recovered as an isolate (88 % to 90 % protein) represents about 75 % of the total protein. More efficient washing of the fababean curd would presumably raise the protein content of the isolate to levels above 90 % protein.

Amino Acid Analyses

Differences in selected amino acid contents between fababeans, soybeans and wheat are shown in Table 5. Of major interest is the high lysine content of the legume proteins and the relatively low levels of methionine. The high levels of glutamic acid and proline which lend characteristic properties to wheat protein are present at considerably lower levels in both fababeans and soybeans.

TABLE 5

Selected amino acid contents (g.a.a./100 g. prot.)

	MET	LYS	ARG	ASP	GLU	PRO
Fababeans. ..	0.64	7.33	10.57	12.88	20.02	4.90
Soybeans. ...	1.39	7.31	9.05	13.97	22.68	5.44
Wheat.	1.23	2.73	4.61	5.38	35.98	10.90

Nutritive Value Studies

Fortification of wheat flour breads with fababean protein concentrate or soybean protein isolate markedly improved the protein quality of the bread (Table 6). Growth rate on the fababean bread and soybean bread diets was more than twice that on the unfortified wheat bread diet. Although weight gain on the fababean and soybean diets did not differ significantly (P 0.05), overall performance tended to be slightly higher for the group fed the fababean bread. Protein efficiency ratio (PER) was appreciably higher for the fababean and and soybean diets. Thus fortification of wheat flour bread with fababean protein concentrate markedly improved the nutritive quality of the bread in spite of a decrease in the sulfur amino acid level relative to protein content in the fababean bread. The results of this study are consistent with the fact that lysine is limiting in wheat flour for growth of young animals and that improvemente of the lysine status of wheat products will improve nutritive quality of these products. Additional studies have shown that lysine is still the limiting amino acid when fababean concentrate is used to replace 9 % of the wheat flour in the mix.

Addition of DL-methionine to diets containing raw fababean resulted in a marked improvement in the growth of rats (Table 7). A level of 0.125 % DL-methionine appeared adequate in a diet containing 10 % crude protein (N x 6.25).

TABLE 6

Comparison of the nutritive value of fababean and soybean supplemented wheat bread by rat assay

Diet	Av. daily wt. gain (g)		Av. daily feed intake (g)		PER[1]	
Wheat bread.	0.58±0.33[2]	a[3]	7.44±1.29	a	0.74	a
Soybean bread.	1.43±0.23	b	9.40±1.35	ab	1.44	b
Fababean bread.	1.67±0.35	b	9.81±0.77	b	1.49	b

[1] Protein efficiency ratio (g body wt gain/g protein consumed).
[2] Mean ± S.D. for 10 rats.
[3] Means followed by different letter differ significantly ($P < 0.05$).

TABLE 7

Effect of methionine supplementation of fababean diets for the growing rat[1]

Diet	Av. initial wt. (g)	Av. daily gain (g)	Av. daily food intake (g)
Casein, 10 % protein.	92.3	3.18±0.72	12.7[2]±1.7
Fababean[3] 10 % protein.	97.6	0.38±0.52	9.8±1.4
Fababean + 0.125 % DL-methionine.	95.0	2.94±0.23	13.1±1.0
Fababean + 0.250 % DO-methionine.	93.1	2.99±0.59	13.1±1.6
Fababean + 0.375 % DL-methionine.	95.0	3.16±0.48	13.8±1.2

[1] 21-day growth trial.
[2] Mean S.D. for 6 rats.
[3] The ground dehulled fababean (31 % crude protein, N

Autoclaving fababean at 115° C for 12 minutes or 35 minutes had no effect on growth (Table 7). Supplementing the fababean diets with 0.250 % DL-methionine again resulted in a marked improvemente in growth. In fact, the present results would suggest that fababean protein is equivalent to casein, provided it is supplemented with methionine.

It is hoped that the data presented in this report will be helpful in assessing the value of fababeans as a source of vegetable protein for both animal and human feeding. If the full potential of this crop is to be realized, much additional research will be required to characterize color, flavor, and potentially toxic compounds found in the seed. Specific tests to characterize the funcional properties of the protein and starch fractions will also be necessary for particular food applications.

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Valor biológico (BV) de doce alimentos peruanos

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ABSTRACT

Protein and BV, by Kjeldahl and NPU/digestibility (Miller & Bender) methods, respectively, were determined in twelve Peruvian foods. Fish Hake meal (for human use, Verrando Process) gave 83 BV; Quinua (Chenopodium Quinua) 50; Cañihua (Chenopodium pallidicaule) 56; Arbol del Pan (Articarpus caudatus) 40.4. These values were the most important. Correlation BV and PER gave: R = 0.82.

INTRODUCCIÓN

La presente comunicación da cuenta del BV determinado en las Chenopodiáceas: Quínua y Cañíhua, granos que se cultivan en las tierras altas del Perú: Árbol del Pan, un fruto de la familia de las Moráceas que se halla en la Selva peruana; variadas Leguminosas bastante conocidas, y de la harina de Merlusa de uso humano, cuyo consumo se va haciendo gradual en nuestro país.

FAO (1970) considera el BV de gran cantidad de alimentos, pero no lo hace con la Quínua, Cañíhua y Árbol del Pan, típicos de la alimentación popular y que traen raíces de tiempos muy remotos, por lo que sus proteínas indudablemente de buena calidad deben ser conocidas en el ambiente internacional.

MATERIAL Y MÉTODOS

La Quínua y Cañíhua estudiadas provienen de las zonas altas del Sur del país (Cuzco y Puno). El Árbol del Pan, de la zona de la Selva. Las leguminosas de las zonas cercanas a Lima. La harina de Merlusa para uso humano elaborada por el proceso Verrando (1972) procede de la negociación de dicho nombre.

Hemos empleado el método de "Utilización Neta de Proteína" (NPU) de Miller & Bender (1955) y "Digestabilidad" (D) para la obtención del BV de los alimentos.

EL NPU se calculó con la ecuación de Bender & Miller (1953):

$$NPU = \frac{B-(Bk - Ik)}{I}$$

donde B y Bk corresponden al N corpóreo total del grupo de animales que recibían el alimento estudiado, y del grupo que consumía la dieta basal sin proteínas, respectivamente; y en donde I é Ik representan el N consumido por los dos grupos de ratas.

La Digestabilidad (D) se calculó con la ecuación de Miller & Bender (1955):

$$D = \frac{I - (F - Fk)}{I}$$

en donde F y Fk corresponden al N excretado por los animales con dieta proteica y no proteica que sirve de testigo, respectivamente é I es el N ingerido de la dieta proteica.

El BV se obtuvo de la expresión NPU de Miller & Bender (1955).

Se emplearon ratas albinas procedentes de nuestros laboratorios, a partir de camadas nacidas con variación de un máximo de tres días (raza originariamente Wistar), se formaron grupos de cuatro ratas siguiendo exactamente a Miller & Bender (1955). Una media de 16 ratas se utilizaron con los alimentos.

Después del destete, las ratas recibieron como alimento la dieta común, administrada a los animales del Bioterio (mezcla Nicovita N.° 8 más cebada) durante 8 días. A continuación las dietas preparadas, iniciándose el experimento en sí, administrando a los animales las dietas respectivas Dieta Basal sin proteínas y dietas proteícas (dieta basal en que cierta parte de carbohidratos se reemplaza por el alimento proteíco, en cantidades adecuadas al porcentaje requerido).

Una vez concluído el experimento se determinó el N corpóreo por el método de Kjeldahl (AOAC, 1960). No se utilizó la ecuación de regresión recomendada por Miller & Bender (1955) para evaluar el N corpóreo porque se trataba de casos en una larga experimentación.

La Relación de Eficiencia Proteíca (PER) se determinó dentro del término en que los animales recibieron la dieta de ensayo, y se aplicó la fórmula: Peso ganado/proteína ingerida.

EXPERIMENTACIÓN

Después de varias tentativas comprobamos que en nuestros animales las etapas de alimentación, de adaptación a la vida individual y el peso, no correspondían a las cifras señaladas por Miller & Bender (1955). Así, nuestras ratas a los 28 días de nacidas sólo alcanzaban de 29 a 31 g. de peso en lugar de los 35 g. de los autores, motivo por el cual se les dejó con sus madres hasta alcanzar el peso necesario. Además, una vez ya separadas en sus jaulas individuales se necesitaron de veinte días para alcanzar los 60-70 g. requeridos para iniciar el experimento.

De otro lado fue necesario reemplazar los carbohidratos de la dieta proteíca comò: almidón de papa, glucosa y arroz, en el caso de alimentos con bajo contenido proteíco como la Quínua, Cañíhua y el Ārbol del Pan.

Punto saltante de nuestro experimento fue la certificación de un 0.63 % de proteína en la dieta basal, cifra considerada en todos los cálculos realizados.

El retiro diario de la comida sobrante, la higiene escrupulosa y la temperatura constante (23° C-0.5) fueron esenciales para obtener resultados reproducibles.

Al final antes de sacrificar a los animales, se esperaba la evacuación intestinal, que ocurría en la noche o en la mañana, a efecto de eliminar una fuente de error.

RESULTADOS

Se presentan en dos tablas. En la Tabla N.° 1 aparece la proteína %, el número de determinaciones de NPU y D, con los respectivos errores estándar, así como el BV y el PER.

Como podrá observarse el contenido proteíco de Quínua (11.0 g %) Cañíhua (14.0 %) y del Ārbol del Pan (17.0 %) es muy apreciable. Desde luego el de harina de Merlusa es el mayor (70.0 %). Los otros alimentos se hallan dentro de los límites conocidos.

El mayor BV corresponde a la harina de Merlusa de uso humano (83), seguido de las lentejas (68) y habas (59). Quínua y Cañíhua tienen como BV 50 y 56 respectivamente, y el Ārbol del Pan 50.5, valores estos últimos que nos indican que se trata de proteínas muy utilizables por el organismo.

PER, que expresa proteína ingerida y peso ganado, da con el BV un factor de correlation $r = 0.82$; $(P > P.01)$.

La tabla N.° 2 nos sirve para comparar NPU, D, BV de FAO (1970), con nuestros valores.

TABLA 1

Proteínas, Utilización Neta de Proteína (NPU), Digestibilidad (D) y Valor Biológico (BV) de alimentos peruanos

	Proteína g %	Replicada	NPU	E.s t.	D	E.s t.	BV	PER
Quinua (Chenopodium quinua). . .	11'0	6	33'6	1'9	67	1'6	50	1'02
Cañihua (Chenopodium pallidicaule).	14'0	4	37'7	2'1	67	2'2	56	1'15
Arbol del pan (Artocarpus caudatus).	17'0	4	28'4	2'5	70'2	3'3	40'4	0'94
Merluza harina Hake meal (human use).	70'1	3	46'0	2'7	58'4	2'8	88'0	3'28
Lentejas. Lentils (Lens sculenta). .	18'7	2	40'6	2'0	59'9	2'5	68'0	1'26
Pallares (Phaseolus lunatus).	19'2	4	32'6	3'5	62	3'1	52'6	1'17
Frijol Castilla (Phaseolus vulgaris).	21'2	2	41'7	1'7	65'1	4'0	64'0	1'59
Frijol canario (Phaseolus vulgaris).	20'8	6	40'4	2'0	60	2'0	67'3	1'83
Frijol negro (Phaseolus vulgaris).	22'1	2	38'9	1'8	56	2'5	69'0	1'79
Arvejas (secas) Peas, dried (Pisum sativum).	21'0	6	35'1	0'8	70	2'2	50'0	1'07
Habas, Broad beans (Vicia faba). . .	21'8	2	34'0	1'3	57	3'2	59'0	0'98
Garbanzo Chick peas (Cisar aretinum).	20'9	4	34'9	4'0	65'8	1'6	53'0	1'00

Cada replicado consiste en un grupo de 4 ratas tratadas como simple grupo.

TABLA 2

Comparación de Utilización Neta de Proteínas (NPU), Digestibilidad (D) y Valor Biológico (BV) y Cuociente Químico (CQ), de algunos alimentos peruanos, con FAO

	NPU	NPU	D	D	BV	BV	PER	PER	CQ
	FAO	Nost	FAO	Nost	FAO	Nost	FAO	Nost	FAO
Quinua.	–	33'6	–	67	–	50	–	1'02	–
Cañihua.	–	37'7	–	67	–	56	–	1'15	–
Arbol del pan.	–	28'4	–	70'2	–	40'4	–	0'94	–
Merluza harina.	65'8	46	93'9	58'4	88'8	88	3'42	3'28	60
Lentejas.	29'7	40'6	85'0	59'9	44'6	68	0'93	1'26	31
Pallares.	–	32'6	–	62	–	52'6	–	1'17	44
Frijol, media.	38'4	40'3	72'8	60'4	58	66'7	1'48	1'74	60
Arvejas.	46'7	35'1	87'6	70	63'7	50	1'57	1'07	37
Habas.	47'8	34	87'2	57	54'8	59	–	0'98	28
Garbanzos.	–	34'9	86	65'8	68	53	–	1'00	40'0

DISCUSIÓN

Dos grupos de métodos permiten evaluar la calidad de las proteínas: biológicos y químicos. El primero se halla representado por el laborioso de Mitchell et al (1945), y el corto NPU de Miller & Bender (1955) que da iguales resultados que el anterior (Campbell, 1963). Este último método al lado de D, de dichos autores, confirmado por Bressani (1970), da el BV. En consecuencia estimamos que hemos empleado entre los muchísimos métodos ideados, el de resultados más ciertos.

El grupo químico, representado por el cómputo proteíco de FAO (1958) o Cómputo Químico (CQ) dado en función de los aminoácidos, no se relaciona exactamente con el BV, tal como indican Bunyan & Price, quienes en 1960, en carne de Ballena de igual composición de aminoácidos hallaron diferente BV. Davidson & Passmore (1966) nos muestran varios alimentos que no guardan la citada relación. Por ello, el CQ ofrece un valor cercano al BV.

Nosotros como ya dijimos, hemos empleado el método del NPU, pero tal vez las dificultades experimentales, que hemos mencionado, no nos han permitido obtener resultados concordantes con FAO (1970), excepto en Merlusa, tal como se puede ver en la tabla N.° 2. De todos modos necesitan una confirmación los alimentos que FAO (1970) no menciona.

También debemos señalar diferencias marcadas entre CS de Quínua y Cañíhua, el cual calculado a partir de las Tablas de Orr & Watt (1957) da 83 y 43, respectivamente; mientras que nosotros hemos hallado un BV de 50 y 56, respectivamente, para dichos alimentos, lo cual dependería de la diferente utilización del N, tal como se dijo antes.

Nuestro PER de Quínua (1.02) y Cañíhua (1.15) es más pequeño que el reportado por Bacigalupo (1970) (1.62 y 2.80, respectivamente) debido tal vez a la extracción de saponinas y a la variedad de composición de los aminoácidos de la proteína de la Quínua (Velázques, 1959).

CONCLUSIONES

Se ha determinado el Valor Biológico (BV) de 12 alimentos peruanos, mediante el método de "Utilización Neta de Proteína" (NPU) y "Digestabilidad" (D) de Bender & Miller (1955). No se utilizó el factor de correlación entre el N corpóreo y agua total, pero se empleó el método de Kjeldahl. Una media de 16 ratas se utilizó para cada alimento. Nuestras conclusiones son:

1) Los alimentos típicos peruanos: Quínua (Chenopodium quinoa) Cañíhua (Chenopodium pallidicaule) y el Árbol del Pan (Artocarpus caudatus), tienen como BV, 50, 56 y 40, respectivamente.

2) La harina de Merlusa para uso humano, elaborado según el proceso Verrando, tiene el más alto BV : 88.

3) El BV de FAO (1970) no concuerda, en diversas legumbres, con nuestros valores.

4) El factor de correlación entre BV y PER fué de $r = 0.82$, $(P > 0.01)$.

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Biological values of beef, textured defatted soy flour, and mixtures of the two

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ABSTRACT

Textured defatted soy flour, commonly called textured soy protein (TSP), lean ground beef, and mixtures of TSP with beef were evaluated for biological value by the PER method. The PER of TSP was increased by the addition of d, l-methionine and the optimum level was about 1.0 % of d, l-methionine added on the basis of the protein content of the TSP. Diets in which the fat levels of necessity were over 8.7 % because of higher fat levels of the beef-TSP mixtures in the diets decreased slightly in PER with increasing fat levels. Casein controls of comparable fat levels were used for each sample. An objective of the study was to correlate chemical scores and available lysine or methionine measurements with PER values of samples, but variations of PER values for the same samples made comparisons difficult and correlations of chemical scores with PER were not attempted in this report.

INTRODUCTION

The commercial availability of textured defatted soy flour, commonly called textured soy protein, and referred to in the following report as TSP is recent. Few reports have appeared in the literature on the biological values of textured vegetable proteins, or mixtures of these products with meats. Since this study was begun, Kellor (1974) reported on the composition and nutritional aspects of defatted soy flour and grits. Hamdy (1974) reported on the composition and nutritional aspects of TSP.

Regulations have been proposed in the United States (USDA, 1974, FDA, 1974) to require minimum PER (Protein Efficiency Ratio) values for certain classes of textured vegetable proteins. The PER method, heretofore used largely in a theoretical sense as a method for ranking of proteins in biological value has sunddenly emerged as a proposed method for establishing and monitoring quantitative standars which must be met to prevent rejection or legal seizure of commercial products. Methods for the quality control of manufactured food

products must be rapid, relatively economical, and reasonably accurate in order to assure that products meet imposed standards and may be placed in commerce within a reasonable time without fear of recall or seizure. The PER method or any other biological method equally expensive and requiring a long time interval is ill suited to these requirements (Alsmeyer, et al. 1974). The method for the chemical score of a protein described by Mitchell and Block (1946) is used by the FAO in calculating chemical scores of proteins (FAO, 1970). In many instances there is reasonably good correlation between PER and chemical score. Measurement of the chemical score of a protein is relatively rapid and less expensive than biological methods currently in use.

One objective of this study was the determination of the PER values of TSP, beef, mixtures of TSP with beef at different levels of fat content, and to ascertain the effects of added d, 1-methionine on TSP. The second objective was a comparison of PER, NPU, chemical score, and lysine and methionine availability as methods for quality control of the protein quality of TSP and mixtures of TSP with beef.

MATERIALS AND METHODS

Samples of TSP from several manufacturers were purchased from commercial channels. D, 1-methionine was added to TSP before texturing. Analysis indicated that at least 95 % of the methionine survived the texturing process. Lean beef was ground twice and freeze dried to less than 5 % moisture. All samples were ground to pass through a 30 mesh screen.

All PER evaluations were carried out by well-known commercial laboratories. These laboratories are coded A, B, C, etc. in this report. PER determinations of beef-TSP mixtures at Laboratory A were made on diets where 67 % of the protein of the diet was supplied by beef and 33 % by TSP. The samples to Laboratories B and F were prepared by incorporating one part hydrated TSP (two parts water, one part dry TSP) with two parts of lean ground beef. This mixture was freeze dried and ground to pass through 30 mesh.

Shell eggs were purchased from commercial channels. The eggs were boiled for 10 minutes, peeled and freeze dried.

Four batches of an experimental baked product fortified to 16 % protein were prepared in different flavors. Formulations varied slightly, only to accommodate changes necessary to change the flavors. Split samples were sent to Laboratories A, B, C and D. Identical formulations of samples 2 and 3 were prepared and sent to Laboratories E and F. Each laboratory was furnished with proximate analyses and asked to determine the PER.

RESULTS AND DISCUSSION

Two commercial samples of TSP manufactured by two suppliers were obtained in which measurements of trypsin inhibitor activity showed at least 90 % inactivation. Corrected PER values reported by several laboratories for their samples varied from 1.68 to 2.33 (Table I). The uncorrected values had a narrower range of 2.01 to 2.34. Other samples varied in value within those ranges but were not cross checked in different laboratories. Corrected values of 2.1 and 2.2 have been reported recently in the literature (Hamdy, Kellor).

Variations in PER values between laboratories is not unique to TSP or products containing TSP, as samples of an experimental food product formulated with added protein to attain a PER of 2.5 or better showed considerable variation in PER between well-known commercial laboratories. These values are shown in Table II.

In the United States, regulations issued by the Food and Drug Administration (FDA, 1973) set the U. S. Recommended Daily Allowance (U. S. RDA) of protein

TABLE 1

Variations between laboratories on two commercial samples of TSP

Sample	PER		Laboratory
	Corrected	Uncorrected	
ANRC Casein. . .	2.5	3.40	A
TSP 1.	1.72	2.34	A
Casein.	2.5	3.23	C
TSP 2.	1.68	2.17	C
Casein.	2.5	2.27	F
TSP 1.	2.33	2.12	F
TSP 2.	2.22	2.01	F

TABLE 2

Variation between laboratories with samples of an experimental processed food product of 16 % protein

Laboratory	Casein PER	PER with Casein Adjusted to 2.5 Sample Number			
		1	2	3	4
A	3.28	2.16	2.30	2.55	2.62
B	2.71	3.04	2.85	2.97	2.80
C	2.95	2.48	2.66	2.75	2.69
D	2.05	2.29	2.47	2.62	2.59
E	2.90	–	2.43	2.57	–
F	2.07	–	2.90	2.87	–

TABLE 3

Effect of the nutritional value of wheat flour on the amount of diet consumed

	Gms.Feed consumed	PER	
		Corrected	Observed
Wheatflour(14.6%protein).	290	0.88	1.10
Wheat flour + Lysine I.	329	1.51	1.89
Wheat flour + Lysine II.	347	1.62	2.03
Casein.	434	2.50	3.13

for labeling purposes at 45 g if the PER of the protein is equal to or better than that of casein, and at 65 g if the PER is less than that of casein. Products for which the packages carry a declaration of the percentage of the RDA of protein per portion on the label based quantitatively on the U. S. RDA of 45 g could appear to be mislabeled simply due to normal variations in PER measurements.

Within each laboratory results on samples of TSP in the same group were

usually consistent. In one laboratory, PER and NPU determinations were done on a series of samples, and correlation was reasonably good as shown in Figure 1. Casein in these studies had a NPU higher than the corresponding PER value, whereas beef was higher in PER than expected from the NPU value. Bender and Doell (1957) found that NPU correlated better with NPR than with PER values. Unfortunately, NPR values were not determined in our studies.

It is common knowledge that the magnitude of corrected PER results often correlates directly with the amount of food eaten. In the case of wheat products, Carpenter (1968) attributes this to the sticky nature of raw wheat protein vs. cooked wheat protein, so the apparent increase in digestibility of cooked wheat protein is due mainly to its increased palatability rather than to a greater biological value of the protein. In our studies, the magnitude of corrected PER values of various materials did not always correlate with amounts of food eaten, but raw white flour from wheat supplemented with lysine was eaten to a greater extent than the flour alone (Table III), which indicates a selectivity by the test animals based on nutritional value as observed by Bender (1956), rather than on palatability alone. Florentino and Broquist (1974) observed similar results when bakers yeast was added to wheat flour.

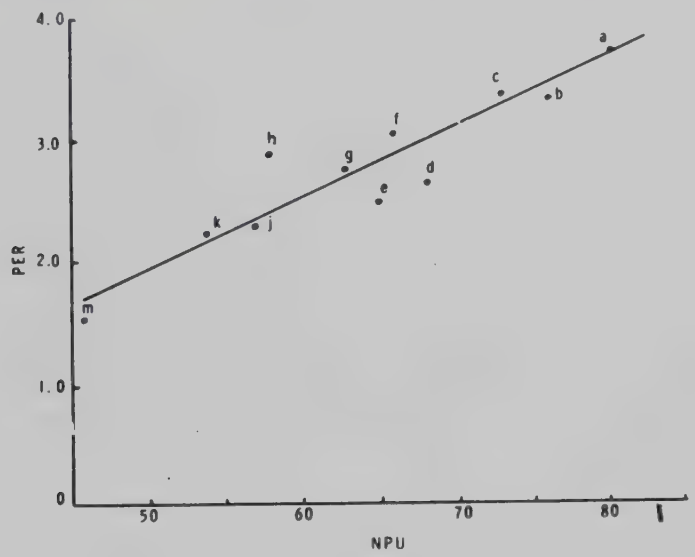


Fig. 1.–Corrected PER and NPU of various materials.

a. Whole egg
b. Egg white
c. Defatted egg yolk
d. ANRC casein
e. Casein, sample 2
f. Beef-hydrated soy (67-33 ratio)
g. Beef-hydrated soy, grilled patty
h. Beef
j. TSP-1
k. TSP-2
m. Defatted soy flour, high anti-trypsin activity.

The responses in biological value of TSP fortified with different levels of d, l-methionine were determined (Table IV). In two laboratories the PER values were increased from about 1.8 to 2.3, the maximum response, with the addition of 1.0 % of d, l-methionine based on protein content of the TSP. Possibly the increase of 0.5 PER would also be obtained in tests by a laboratory in which the PER of the unfortified TSP was higher than 1.8.

The PER values of lean, ground beef extended with 33 parts of hydrated TSP

TABLE 4

Effects on PER of d, l-methionine added to TSP

Sample	PER		Laboratory
	Corrected	Uncorrected	
1. Casein control a.	2.5	2.70	A
TSP a.	1.83	1.97	A
TSP + 1 % d, l-meth.*.	2.30	2.47	A
2. Casein control b.	2.5	3.12	A
TSP b.	1.69	2.11	A
TSP + 0.5 % d, l-meth.	1.87	2.33	A
TSP + 0.75 %d, l-meth.	2.08	2.60	A
TSP + 1.0 % d, l-meth.	2.29	2.86	A
TSP + 1.25 % d, l-meth.	2.37	2.95	A
TSP + 1.40 % d, l-meth.	2.29	2.86	A

*** Percentage of d, l-methionine based on protein content of TSP.**

to 67 parts of beef were determined in different laboratories. In laboratories A and B the extended beef was slightly lower in biological value than the beef. In laboratory F the beef extended with TSP was of slightly higher biological value than beef alone. Hamdy reviewed the work of Kies and Fox (1971) and Korslund et al (1973) in which it was found that adults fed protein at levels too low to maintain positive nitrogen balance showed better response in nitrogen retention to beef than to TSP. TSP plus d, l-methionine or TSP plus beef up to 30 % replacement of beef with TSP, showed nitrogen retention only slightly below that obtained with beef alone. The reports of Hamdy and Kellor contain a great deal of detailed information on the composition and nutritional aspects of defatted soy flour and grits and of TSP. Kellor reports a corrected PER for defatted grits of 2.19. Hamdy reports PER

TABLE 5

PER of mixtures of Beef (67 %) and TSP (33 %)*

Sample	PER		Laboratory
	Corrected	Uncorrected	
Casein.	2.50	3.38	A
Lean Ground Beef.	2.67	3.61	A
TSP a - Beef.	2.29	3.09	A
Casein.	2.50	3.10	B
Beef.	2.88	3.57	B
TSP c - Beef.	2.63	3.26	B
TSP - Beef, grilled patty.	2.56	3.17	B
Casein.	2.50	2.27	F
Beef.	2.90	2.63	F
TSP c - Beef.	3.04	2.76	F
TSP - Beef, grilled patty.	2.79	2.53	F

*** Hydrated, 2 parts water to 1 part TSP.**

values for TSP of 2.10 and 2.37 for beef. Hamdy's report described experiments with animals, in addition to PER studies, to evaluate the biological value of TSP. In general, he concluded that TSP as used in the United States School Lunch Program and in commerce is a nutritional food of good quality.

Turk et al (1973) carried out nitrogen balance studies on spun protein foods consisting of soy protein as the principal source of dietary nitrogen. They concluded that spun fiber foods of soy represent a high quality of protein for teenagers and adults.

The official PER method of the U. S. AOAC (Derse, 1960) stipulates the inclusion of 10.0 % of protein (N times 6.25) and 8.7 % of fat in the diets fed the test animals. In commerce, mixtures of TSP and of ground beef or other meats are often used in which the fat levels are too high for diets containing these products to be adjusted to 8.7 % fat commensurate with a protein content of 10.0 %. In results reported previously in this paper, all PER values were determined on diets containing 10.0 % protein and 8.7 % fat. The diets with higher levels of fat were prepared from the same lean beef or beef-TSP mixtures by addition of rendered beef fat at the expense of carbohydrate. For the determination of the PER of these mixtures, casein control diets were used in which the fat levels were adjusted to the same levels as those of the hydrated TSP-beef mixtures. The PER values of the beef extended with TSP decreases with an increase in fat content of the mixture (Table VI), but the magnitude of the decrease is not great.

TABLE 6

Effect of fat on the PER of mixtures of beef (67 %) with TSP (33 %)*

	% Fat	Gms. food consumed	PER, Lab. A	
			Corrected	Uncorrected
Beef - TSP.	8.7	461	2.29	3.09
Casein.	8.7	464	2.50	3.38
Beef - TSP.	15	457	2.21	3.00
Casein.	15	461	2.50	3.40
Beef - TSP.	21	450	1.97	2.66
Casein.	21	437	2.50	3.38

*** TSP hydrated with 2 parts of water to 1 of TSP.**

The U. S. Food and Drug Administration (FDA 1974) has proposed a minimum PER value of 2.0 (80 % of that of casein corrected to 2.5) for plant protein products used as extenders or replacements for meat, seafood, poultry, eggs, or cheese if they are not to be labeled "imitation". Our data shows that any value set as a minimum greater than 1.7 could result in rejection of commercial lots of TSP simply because of normal variations in PER resuts among laboratories. It would be necessary to fortify commercial TSP with d, l-methionine in order to ensure levels of PER of 2.0 or greater. The food additive order permitting methionine requires however, that a PER equivalent to that of casein be achieved in the finished ready-to-eat food. (FDA, 1972).

There may be several reasons that PER values vary, but the corrections of values obtained to the value of 2.5 for a casein control may be a principal reason. It was noticed that in general lower corrected PER values in textured soy protein and on processed food products correlated inversely with actual values obtained on

casein controls. If values obtained on casein controls were substantially over 3.0 PER, corrected values of PER of samples from the same lot were usually lower than expected. Conversely, if casein controls were below 3.0 PER, corrected values of protein analyzed were usually closer to expected values. A probable reason for this correlation is that the technology of preparing good, undamaged casein has advanced considerably in the last few years. Commercial caseins prepared now by spray drying are of much better quality than caseins of a few years ago and presumably of better biological value. Laboratory B (Table VII) reported an increase in uncorrected values for ANRC casein in the last few years, Laboratory A reported an increase but did not provide figures. Laboratory C had fairly constant figures over the last six years. It might be appropriate to correct modern casein controls to 3.0 PER instead of 2.5. At any rate, a new collaborative study between laboratories may be in order to determine the corrected value of the modern casein controls that best fits average PER values obtained.

TABLE 7

Increase in the PER of ANRC casein in the last several years in commercial laboratory B

Year	Protein level %	No. of tests	Average PER	Range
1969-72.	9	10	2.78	2.19-3.07
1972.	9	8	2.76	2.28-3.41
1973.	9	9	2.98	2.62-3.53
1973.	10	7	3.15	2.52-3.71
1974.	10	9	3.23	3.03-3.54

A possible affirmation of the increased biological values of casein controls in recent years might be inferred from PER values obtained on eggs in the last year or two compared to values reported in previous years. In Table VIII are shown data which confirm that corrected values of eggs obtained since about 1968 are lower than those reported prior to that time, but there is little change in uncorrected values. Although Laboratory C had reported fairly constant uncorrected values for ANRC casein over the last six years in Table VIII it is shown that the corrected values for whole egg in Laboratory C reported in 1973 decreased from the corrected PER values reported in 1958.

There is little or no processing damage to protein in the preparation of TSP, as the textured product is heated only enough to inactivate natural anti-metabolites present in the soybean. Textured or steamed (autoclaved 15 minutes at 15 psi) defatted soy flour in which the trypsin inhibitors are inactivated have the same biological value and show no protein damage. A control of the biological value of TSP is really unnecessary, as appreciable damage to protein in processing would be accompanied by changes in color, flavor, and loss of hydration which would render the product of poor quality as a meat extender. The biological value of TSP is governed by the amino acid pattern of the defatted soy flour from which it is derived, omitting effects of growth inhibitors, and not by the texturing process.

Rackis (1974) in a review of the work of Kakade, Liener, and others stated that a reduction of trypsin inhibitor activity by 79 % results in optimum biological value of soy protein. The extent of reduction of trypsin inhibitor activity might be used as a control for TSP, but the practical value of this measure is in doubt, since Nordal

TABLE 8

PER of egg determined recently compared to older values

Sample	PER		Place	Date
	Corrected	Uncorrected		
Casein.	2.5	2.76	Lab. C	1958
Whole Raw Egg.	3.45	3.80	Lab. C	1958
Whole Fried Egg.	3.51	3.86	Lab. C	1958
Casein.	2.5	3.25	Lab. C	1973
Whole Fried Egg.	2.98	3.87	Lab. C	1973
Whole Boiled Egg.	2.98	3.87	Lab. C	1973
Whole Omelet.	3.02	3.92	Lab. C	1973
Casein.	2.5	3.28	Lab. A	1973
Whole Boiled Egg.	2.94	3.85	Lab. A	1973
Casein.	2.5	2.27	Lab. F	1974
Whole Boiled Egg.	3.74	3.40	Lab. F	1974
FAO Study #24*				
Whole Egg.	3.50	–	Canadian	1959
Whole Egg.	3.50	–	Food & Drug	1959
Whole Egg.	3.57	–	–	1960
Whole Egg.	4.01	–	–	1963
Whole Egg.	4.74	–	–	1964
Whole Egg.	4.20	–	–	1965
Average of FAO Values.	3.92			

*** Values reported in FAO Nutritional Study #24, "Amino Acid Content of Foods and Biological Data on Proteins", page 180 (1970).**

TABLE 9

Chemical scores of proteins of beef and soy

Reference protein	Beef	TSP Hydrated*	Blend of Beef (67 %) hydrated TSP (33 %)
FAO+.	103 Val.#	85 S.	97 Val.
Whole Egg.	67 S. (Sulfur)	52 S.	62 S.
Human Milk.	53 Try.	87 Try. Leu.	63 Try.

*** One part TSP, two parts water.**
+ FAO Provisional Adult Pattern, 1970; Egg and milk, values FAO (1970).
Limiting amino acid.

and Fossum (1974) found that in the presence of meat, soybean trypsin inhibitors were easily inactivated by exposure to heat less than that encountered in cooking meats extended with TSP.

Should it be deemed necessary to require a measure of biological value as a standard for commercial preparations of textured vegetable proteins, the imposition

of a standard based on an inflexible, realtively high PER value would raise serious problems. For quality control of the biological value of textured vegetable proteins a rapid, economical, reasonably reproducible method is needed. The PER determination or any other equally cumbersome method is unsuited in all respects. It must be kept in mind that a control method is not necessarily an absolute method to determine precise biological values, but rather as a method which is applicable to control needs and which correlates reasonably well with the official method.

It is of interest that the chemical score of TSP protein is higher than that of beef compared to human milk rather than to whole egg or the FAO pattern for adults (Table IX). Since the PER of proteins is dependent on the growth response of weanling rats it would also be of interest to compare the chemical scores of TSP calculated from the amino acid profile of human milk and of rat milk. We have been unable to find the amino acid content of rat milk in the literature in order to make that comparison.

This series of studies was intended in part to correlate PER values of TSP and beef extended with TSP with chemical score, which is a simpler method suited for quality control monitoring. The variability of PER values measured on the same samples in different laboratories made correlation difficult and it was not attempted in this report. Since the limiting amino acids of TSP are the sulfur containing amino acids an effort to develop reasonably accurate simple methods for the determination of total sulfur containing acids and of biologically available methionine in a protein is worth consideration. Coupled with specific methods for sulfur amino acids, a system based on chemical score might be appropriate to measure the biological values of TSP and other low sulfur-protein sources.

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Sources of food nutrients in India

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ABSTRACT

Some lesser-known sources of dietary nutrients in India are highlighted. Two cereals deserve attention. The millet ragi (*Eleusine coracana*) carries 7 percent of a biologically wellbalanced protein, and is exceptionally rich in calcium (350 mg/100g) and high in iron, thiamine, riboflavin and niacin. Bajra (*Pennisetum typhoideum*) has 11.5 percent of protein rather deficient in lysine, but not in sulphur amino acids. Pulses of high lysine content are the horse gram (*Dolichos biflorus*), red gram (*Cajanus cajan*), field bean (*Dolichos lablab*) and kidney bean (*Phaseolus vulgaris*). Balanced food formulations using these ingredients require a minimum of skim milk powder to ensure high protein quality. Among fruits, the flesh of the seetaphal (*Annona squamosa*) has an exceptional 14 percent of glucose and high levels of thiamine, riboflavin and niacin, while the common sapota (*Achras sapota*) is exceptional in that 5.4 percent of its total sugar content of about 15 percent is present as galactose. The green leaves of numerous Indian vegetables like spinach, amaranth, colocasia, drumstick, mustard and fenugreek are practically concentrates of vitamins A, C and B-group, and of calcium and iron.

INTRODUCTION

In India, a vast programme of supplementary feeding for vulnerable segments of the population, in particular preschool children, school children, pregnant women and lactating women, is now being organised and operated by its Government. The criteria laid down are that a serving of the supplementary food should carry 300 calories and about 10-12 grams of protein, while lying below a certain price. Foods used in these feeding programmes are generally cereal —oilseed/pulse— milk powder blends. Some are imported from abroad, such as corn-soy-milk (CSM) and wheat-soya-blend (WSB), and others (like Balahar) are produced in the country using local ingredients. Blends in India usually consist of rice or wheat as the cereal, while the protein source is generally a whole oilseed or oilseed protein like the groundnut and sesame, with sometimes an added pulse, usually the Bengal gram (*Cicer arietinum*). As high a protein quality as possible is

sought to be attained by judicious blending of ingredients; a protein efficiency ratio of 2.0 is usually possible.

In India, as in most underdeveloped countries, there are food ingredients of exceptional nutritive value which are not as widely employed as they should be in the daily diet. Knowledge of such food ingredients among doctors, dietitians, social workers and the public could well stimulate their wider use. Use of such ingredients in the blends required for welfare feeding will be fairly easily achieved once known. Manufacturers of processed foods may also be able to employ these sources with advantage.

RESULTS & DISCUSSION

The analytical data in this paper are drawn mostly from the authoritative volume entitled "Nutritive value of Indian foods", published by the Indian Council of Medical Research, New Delhi (Gopalan et al., 1971).

Staples

Wheat and rice have been used almost exclusively in India for the preparation of food blends for the feeding programmes. Two other lesser-known cereals also deserve special mention. *Eleusine coracana*, commonly known as ragi in the South, and as nachna in Western India, is raised to the extent of 2 million tonnes annually in the country, constituting about 2 percent of the total foodgrain crop. It consists of purple or brown globules about one mm in diameter. The fibre content is about 5-6 percent, the lowest in any foodgrain crop, and the skins are easily removed by wetting, sprouting, drying and gentle rubbing or pounding. Such skin removal reduces the fibre level and leads to a flour which is uniform and not mottled in colour. Flavour and taste are exceptionally pleasant. Sprouting also enhances the vitamin C content and improves digestibility of the starch and protein.

Nutritionally ragi has several attractive features. The protein content varies from about 6-11 percent, averaging 7.3 percent. The protein is of exceptionally good quality, without a single limiting amino acid relative to FAO reference protein. Whereas in rice protein both lysine (230 g/kg of nitrogen, FAO 270 g) and methionine + cystine (240 g, FAO 270 g) are marginal, the proteins of ragi are adequate in lysine (270 g) and exceptionally rich in the two sulphur-containing amino acids (350 g). Table 1 lists these and other values. The calcium level is exceptional at 344 mg/100 g, and habitual ragi eaters in Karnataka and Maharashtra States are believed to ingest as much as 2 g of the mineral a day (WHO, 1962). The quantities of other nutrients in ragi all give it nutritional significance as a food ingredient; these include (in 100 g of ragi) iron 17 mg, vitamin A 18 mcg, thiamine 0.42 mcg, riboflavin 0.10 mcg and niacin 1.1. mcg. As an ingredient of baby foods, whether home-made or manufactured, ragi is of special value as the most practical and inexpensive source of calcium when milk is out of reach. A recipe developed by the National Institute of Nutrition, Hyderabad, India comprises mixing together the roasted powders of deskinned ragi 60 g, mung dhal 15 g, decuticled groundnuts 10 g and dehusked sesame seed 5 g, with skim milk powder 15 g. A tablespoon of the mixture stirred up with warm water suffices for one feed for a young child.

A second cereal deserving of wider use is bajra (*Pennisetum typhoideum*, pearl millet) of which some 6.5 million tonnes are grown annually in India. It is advisable to remove fibre by dehusking after roasting. The fat content (5.0 percent) is exceptionally high, as is the protein content (11.6 percent). Such high protein levels in the dietary cereal component will mean that less protein supplement will be required for a proper balance. Table 1 shows that the methionine + cystine levels

TABLE 1

Some less-known protein sources

Source	Protein (%)	Grams per kilogram of nitrogen: Lysine	Grams per kilogram of nitrogen: Methionine + cystine
FAO reference protein.	(100)	270	270
Cereals			
Ragi *(Eleusine coracana)*.	7.3	270	350
Bajra *(Pennisetum typhoideum)*.	11.6	190	270
Pulses			
Horse gram *(Dolichos biflorus)*.	22	520	200
Red gram *(Cajanus cajan)*.	22	480	120
Bengal gram *(Cicer arietinum)*.	21	440	160
Field bean *(Dolichos lablab)*.	24	500	120
Kidney bean *(Phaseolus vulgaris)*.	23	460	100

just about equal that of FAO reference protein standard (270 g kg of nitrogen), but lysine (190 g) is rather below reference level (270 g), and supplementation with lysine-rich protein material will be required to ensure the best protein quality. Fortunately several common Indian pulses are rich in lysine.

Pulses

A large number of pulses and grams are in common use in the country. Most contain between 20 and 25 percent protein. Yet in manufactured foods for use in the feeding programmes, it is oilseed proteins that are always thought of. In practice this has been groundnut protein, use of which has been bedevilled by the aflatoxin problem, especially in foods meant for children. Soybean production has made only limited progress in India. Of the other proteins, that of the Bengal gram (*Cicer arietnum*), which is by far the most widely grown of the pulses, is the only one that has found some use in food formulations. Yet a glance at Table 1 will show the potential of several other common pulses. All are exceptionally rich in lysine (440-520 g/kg of nitrogen), thus complementing the cereal components excellently, but are deficient in methionine + cystine. T of boost protein quality, som proportion of sesame flour or skim milk powder in the blended formulation is therefore desirable.

In the near-famine conditions that occurred in Maharashtra State in the winter of 1972/73, some of these pulses were indeed pressed into service in making formulated food mixes for relief distribution. Table 2 shows a number of protein-concentrate formulations which were developed at the Hindustan Lever Research Centre, Bombay (Menon, 1974). These contained about 40 percent protein, and when blended at an 18 percent level with 42 percent wheat flour, 30 percent jaggery (brown sugar) and 10 percent fat, yield a food mix which conformed to the nutritional and price criteria earlier outlined. This example needs to be better known, and emulated more widely.

Vegetables

Green leafy vegetable tops are familiar articles of food in India. Particularly in the northern states of Punjab, Haryana and Madhya Pradesh, where nutritional status is the highest in India, average consumption of leafy vegetables exceeds 50 g a day. Products commonly consumed are the leaves of the spinach and of the

TABLE 2

Protein formulations
Protein level: About 40 percent
PER = Protein efficiency ratio
PER of casein = 3.0
All figures as percentages by weight

A. Using whole groundnuts

	1	2	3	4	5
Groundnuts	30.5	25.3	15.0	36.4	20.8
Sesame seed	19.8	18.8	20.0	–	–
Soyabeans	–	7.4	30.0	15.6	31.2
Bengal gram	25.1	14.0	15.0	20.0	20.0
Horse gram	24.6	–	–	17.0	16.2
Black gram	–	22.8	–	–	–
Skim milk powder	–	11.6	20.0	10.9	11.7
Lysine	–	0.1	–	0.1	0.1
PER	2.5	2.3	3.1[a]	2.1	2.3

B. Using groundnut meal

	6	7	8
Groundnut meal	51.9	37.2	29.0
Sesame meal	–	16.6	26.0
Bengal gram	20.0	35.5	17.5
Horse gram	18.0	–	17.4
Skim milk powder	10.0	10.6	10.0
Lysine	0.1	0.1	0.1
PER	2.0	2.4	2.5

C. Using no groundnut

	9	10	11
Sesame seed	20.0	23.5	–
Soyabeans	–	35.3	50.0
Black gram	67.0	–	–
Bengal gram	–	17.7	20.0
Horse gram	–	–	20.0
Skim milk powder	13.0	23.5	10.0
PER	2.8[b]	3.3	2.5

[a] Lysine (0.1 percent), if added, raises the PER to 3.3.
[b] Methionine (0.1 percent), if added, raises the PER to 3.4.

rape-mustard family, which have a tang which is much relished. Table 3 shows a number of common Indian leafy vegetables and their contents of several vitamins and minerals, along with the recommended adult daily allowances for the latter. The values listed for the B-group vitamins are the totals for thiamine, riboflavin and niacin. So high are most of the nutrient levels that these sources can almost be considered concentrates of these vitamins and minerals. In recommending consumption of these foodstuffs, some reservations have been expressed in respect of their contents of fibre and oxalates. Srikantia (1973) has reviewed the evidence. Fibre contents are well bellow a maximum of 2 percent, and the oxalate contents, though slightly high in spinach and amaranth (650 and 750 mg/100 g), are no nutritional drawback to consuming the 125 g a day recommended for adults by the Indian Council of Medial Research in a balanced diet.

Fruits

Two fruits, both of fairly common occurrence, invite comment because of

TABLE 3

Vitamins and minerals in Indian green leafy vegetables. The figures show the quantities present in 100 g of edible portion

Vitamin or mineral	Traditional rich sources		Spinach	Amaranth	Colocasia leaves	Drumstick leaves	Mustard leaves	Fenu-greek	RDA for adult male
Vitamin A mcg	Carrots	1800	5600	5500	10000	7000	1300	2300	750
	Butterfat	1600							
	Papaya	600							
Vitamin B-group mcg	Egg	1800	800	1500	1600	1000	900	1150	25
	Mutton	800							
	Papaya	500							
Vitamin C mg	Amla	600	30	100	12	220	60	50	50
	Lemon juice	40							
	Papaya	50							
Calcium mg	Prawn	300	70	400	220	450	370	400	400-500
	Pomfret	200							
	Milk	200							
Iron mg	Liver	6	10	25	10	7	12	17	20
	Sardine	6							
	Mutton	3							

certain exceptional features. The sapota or cheeku (*Achras sapota*, family *Sapotaceae*) is a dark-brown ovoid or globular fruit, about 5 cm long. It has a thin skin, usually discarded, from which the sweet, soft, deep-brown flesh in easily detached. Two large, flat, hard, shiny black seeds are present. The edible portion is slightly fatty (2 percent) but not otherwise rich in nutrients (per 100 g: calcium 28 mg, vitamin A 30 mcg, ascorbic acid 6 mg). Of special interest is the carbohydrate component, comprising 23 percent, of which about 15 percent appears to consist of various sugars. About 5.4 percent of this is reported (Venkataraman and Reithel, 1958) to consist of galactose, which is not a commonly-occurring sugar. Galactose is required for galactolipid formation in growing tissues, and the use of the fruit in artificial child feeding, especially during weaning, deserves consideration. Strained or pureed baby foods based on the sapota, suitably fortified with such vitamins as ascorbic acid, may have possibilities both in internal and in export markets.

The seetaphal or sharifa (*Annona squamosa*) is a green globe up to 8 cm in diameter with well-defined knobs on the outer surface. Each knob corresponds to a wedge of flesh enclosing one large seed. The pulpy flesh is soft and sweet, and filling the spaces between the wedges is a sweet, rather gritty paste. The fruit grows wild in several states, notably Andhra Pradesh, and is sold comparatively cheap. The cultivated varieties are big in size and have large knobs and therefore fewer seeds and more pulp, and carry a delicious flavour. The nutrient qualities are quite exceptional. Glucose, which in several fruits constitutes about 5-8 percent, is as high as 14 percent in the seetaphal, and calcium is at the exceptionally high level of 400 mg/100 g. The fruit is also rich in other nutrients; per 100 g of edible portion,

these are: thiamine 70 mcg, riboflavin 170 mcg, niacin 1300 mcg and ascorbic acid 37 mg. One fruit a day would meet almost the total requirements of calcium and ascorbic acid. Greater production and distribution of this delicious fruit needs to be encouraged. Manufacture of strained pulps for babies would bear investigation.

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The wet milling of coconut kernel

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ABSTRACT

The performance of a colloid mill in wet milling of coconut kernel has been studied with reference to:

(a) The quantitative efficiency of release of oil in the form of O/W emulsion; and

(b) The size distribution of oil globules in the emulsion and of particles in the solid residue.

The results in conjunction with those of an earlier study of the performance of a wedge cutter/die plate mill provide the basis for development of an improved wet milling system.

INTRODUCTION

In recent years the O/W emulsion which can be extracted from coconut kernel has become a subject of considerable technological interest. Commercial production of the emulsion has commenced, or is projected, in a number of countries where it is a well established item of diet and there may be scope for the establishment of a substantial export trade. In addition, the emulsion is a potential alternative to copra as an intermediate in the commercial production of coconut oil; the realisation of this potential would conserve vast quantities of food protein which are degraded and rendered unfit for human consumption by conventional methods of oil production. The numerous published and patented wet processes for production of oil and edible protein products have been reviewed by Rajasekharan and Sreenivasan (1967) and Dendy and Timmins (1973) amongst others. None of these processes are in commercial use at present and it seems probable that none is yet commercially viable. An economic evaluation of the process developed by Dendy and Timmins (1973) has been reported by Edmunds et al (1973) and this indicated that the process was not competitive with traditional methods as a means of oil production. Work is continuing with a view to improving the competitive position of the process. The present study relates to the wet milling operation which forms the first stage of the process.

EXPERIMENTAL

The colloid mill employed was a Premier Colloid Mill Model 84 fitted with 84 mm diameter carborundum discs. The rotor was driven at 8,000 rpm.

The wedge cutter/die plate employed was a Mincemaster Model F246 with a cutting blade driven at 3,000 rpm; it is referred to as the WCDP mill in the following text.

The homogeniser employed was an Ormerod QP valve type Series 4, Size 3, type VY3.

Standard procedures for washing and pressing solid residues were similar to those reported by Dendy and Timmins (1973).

AOAC (1970) methods were used for determination of moisture and fat content of feedstock, residues and emulsions.

Particle determinations for residues were made by means of a wet sieving technique in which 25 g of pressed residue was washed with copious quantities of water on a 1400 μm screen of 203 mm diameter. The oversize was transferred to a tared filter paper and dried at 60° C. The process was repeated sequentially for the undersize materials with screens down to 106 μm.

RESULTS AND DISCUSSION

The oil bearing tissue of coconut kernel consists of parenchyma cells in which oil is present as discrete globules; the cells are elongated and of polygonal cross section —generally hexagonal or pentagonal— close packed and oriented at right angles to the testa (Manzanilla (1953), Timmins (1970)). In the sections examined by Timmins the cells ranged in length from 70-700 μm and in width from 15-80 μm.

Is it necessary —in order to obtain optimal release of oil and protein— to grind the kernel to a fineness corresponding to this cell size distribution, so that the solid residue consists of individual ruptured cells and small aggregates of such cells; or does release occur readily by diffusion and leaching from larger aggregates? This is the fundamental question in wet milling.

Dendy and Timmins (1974) have shown that for a particular milling system —a two stage WCDP mill— there is a strong correlation between the percentage oil release during milling and the particle size distribution in the solid residue within the range of cell dimensions. Release of oil corresponding to extractions of up to 95 per cent were obtained with this system; but 95 per cent extraction entailed a size reduction such that 78 per cent and 30 per cent by weight of the solid residue consisted of particles of diameter less than, respectively, 500 μm and 250 μm.

These results provide convincing evidence that high degrees of cell rupture must be achieved during milling. It is arguable, however, that the particle size distributions in the residues are characteristic of this particular milling system; and that, for other systems, corresponding degrees of cell rupture and oil release may be associated with a different residue size distribution pattern.

To elucidate this point a systematic study has been made of the performance of a disc type colloid mill in wet milling operations. Although this type of mill has been used in a number of wet processes —eg Sugarman (1956), Hagenmaier et al (1971)— there is no previous published account of such a study. The results quoted in Table 1 are typical of those obtained. They derive from a trial in which the initial feedstock consisted of coconut kernel diced to 5 mm and slurried with water in the ratio 1 : 5 by weight. Milling operations were carried out sequentially at 50° C with residues subjected to (a) standard washing and pressing procedures; and (b) standard pressing without washing at each stage. It will be seen that emulsion extractions equivalent to oil release approaching 100 per cent are obtainable by use of the colloid mill.

TABLE 1

Colloid Mill Setting ($mm \times 10^{-3}$)	Overal Oil Extraction (%)	
	(a)	(b)
889	77.9	81.2
635	89.0	87.6
381	92.9	90.7
254	94.5	93.3
127	96.9	94.7
25.4	98.5	96.1
12.7	–	97.8

In Figure 1 the particle size distributions in pressed unwashed solid residue are shown for the various stages of milling. They show that for particular levels of oil release the degree of size reduction is far less than that produced in the two stage WCDP mill system. At 95 per cent extraction, for example, 21.8 per cent and 5.5 per cent respectively of the residue consists of particles which pass a 500 μm and a 250 μm screen. Size reduction in colloid milling is therefore markedly less than that obtaining in WCDP milling for equivalent oil extractions.

Apart from the wasteful use of energy associated with excessive size reduction there are practical objections to such reduction in a wet milling process; for subsequent stages of processing are complicated if the emulsion produced in milling has a high content of very fine solids. A further possible complication must also be considered. It has been widely accepted that grinding of coconut kernel to sub-cell

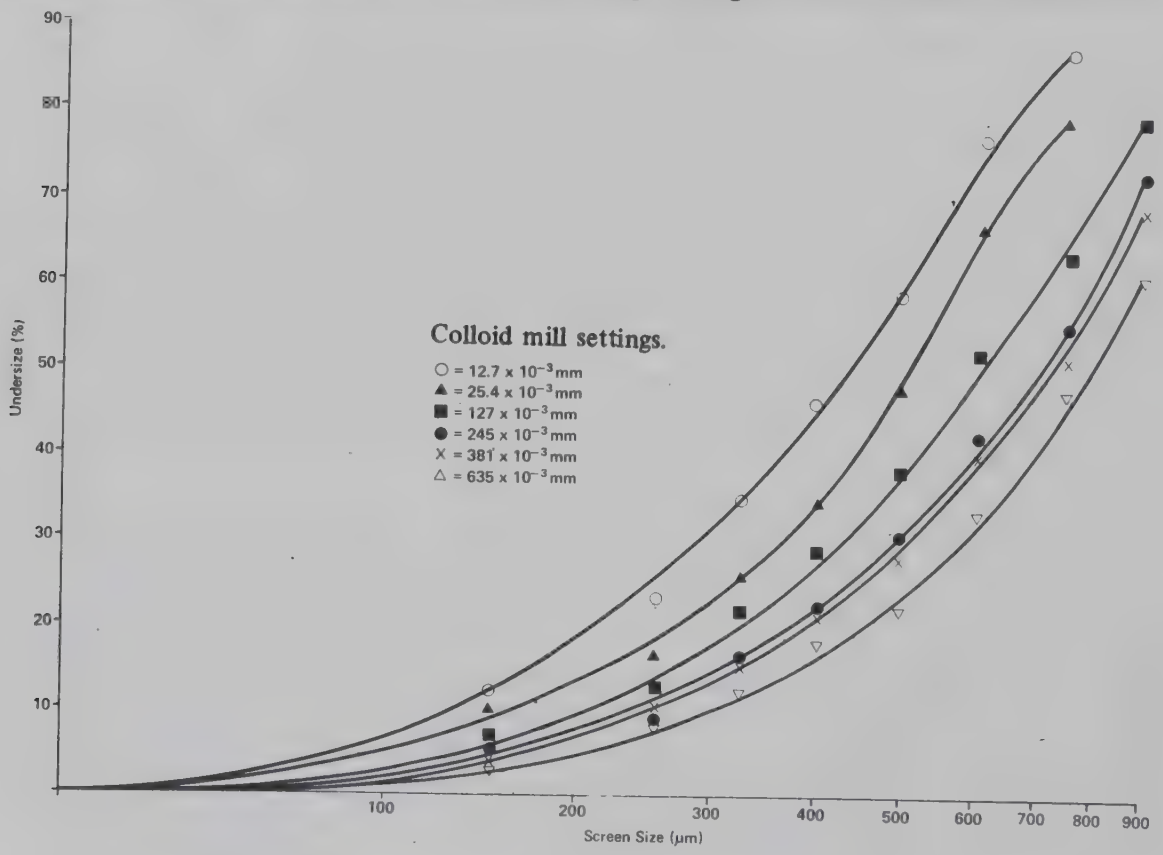


Fig. 1.

dimensions leads to reduction of oil globule size in the emulsions released. This could be an advantage if the ultimate product is to be a stabilised cream, and a corresponding disadvantage if the objective is coconut oil production. In the latter case, if the globule reduction hypothesis is correct, it might be advantageous to employ a milling system which is not the most efficient in terms of quantitative release of oil but which yields emulsions in which oil globules are relatively large. Jefferies (1963) was guided by this consideration in his selection of the rod mill as the unit of choice for wet milling, and it seems likely that processes involving roller mills and expeller presses were similarly conceived.

Hagenmaier et al (1971) conducted photomicrographic studies at x440 which provided evidence in favour of the globule reduction hypothesis. This must be interpreted with caution, however, because such studies are unlikely to account accurately for globules below 1 μm in diameter; they are thus likely to yield misleading results both as to the size range of globules and the pattern of distribution within this range. A more reliable indication can be obtained by centrifugal sedimentation studies since it is now possible to account for globules of diameter above about 0.25 μm by this means (Sherman 1973). The method is particularly useful in comparative studies, and it has therefore been applied to emulsions prepared as follows:

A Coconut kernel grated gently using a hand grater, and the emulsion extracted by hand pressing of the grated mass in a woven terylene bag. This is a simulation of the domestic method of preparing coconut milk used in Thailand and elsewhere.

B Coconut kernel grated by hand and then milled in a colloid mill set at 254×10^{-3} mm in a 1 : 5 slurry in water at a temperature of 50° C.

C As in B, but with the colloid mill set at 12.7×10^{-3} mm.

D The emulsion from A homogenised at 50° C by a two stage process at respectively 210.9 kg/cm^2 and 35.2 kg/cm^2.

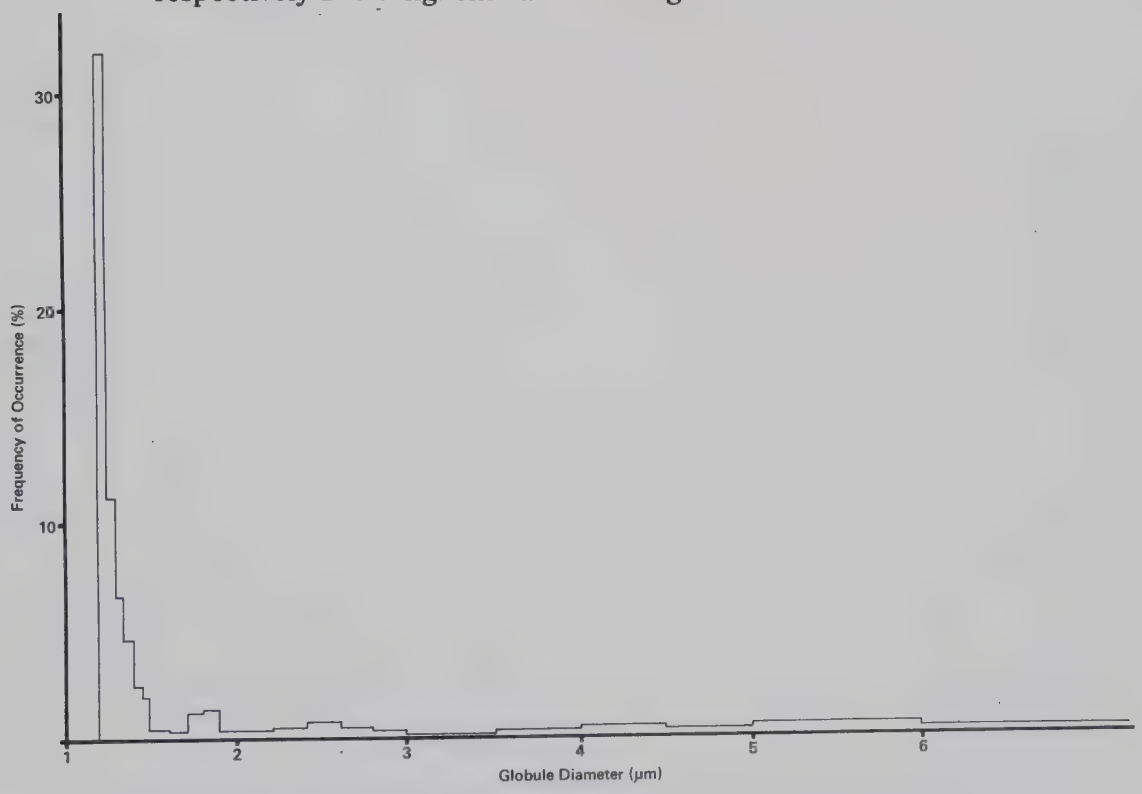


Fig. 2.–Globule size distribution. A. Manually prepared emulsion.



Fig. 3.–Globule size distribution. B. Emulsion from colloid mill at 25.4×10^{-3} mm setting.

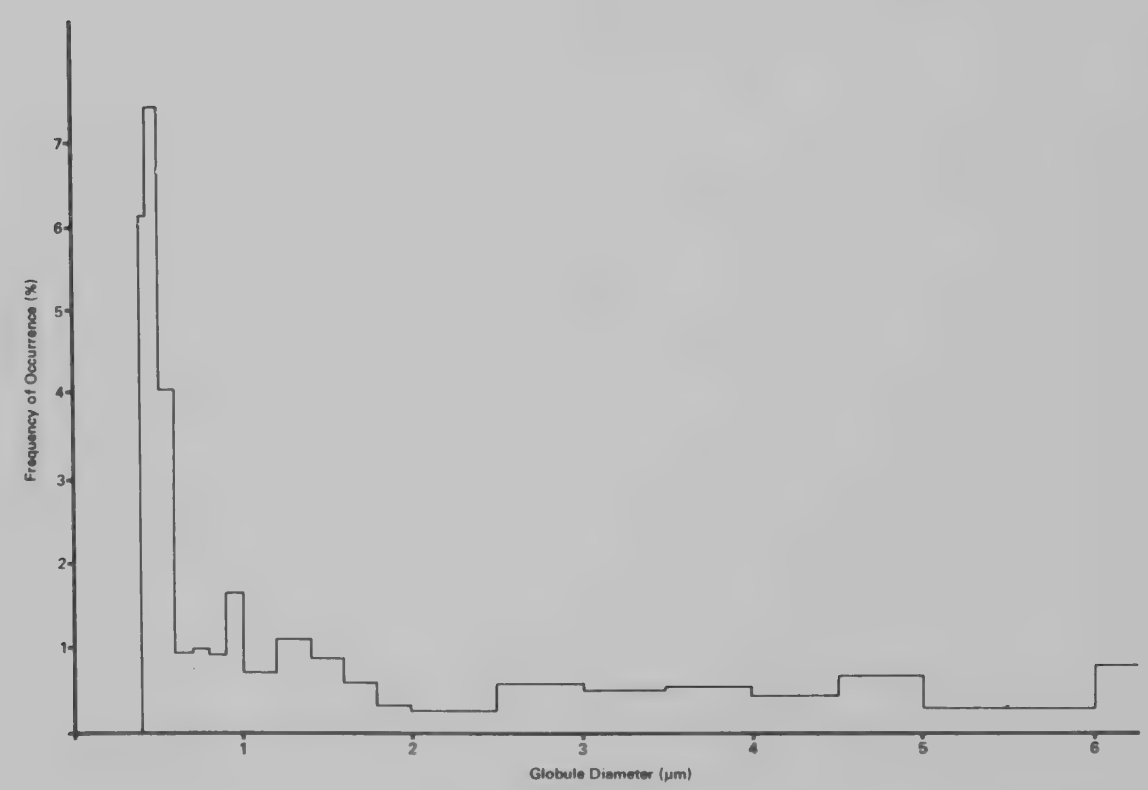


Fig. 4.–Globule size distribution. C. Emulsion from colloid mill at 12.7×10^{-3} mm setting.

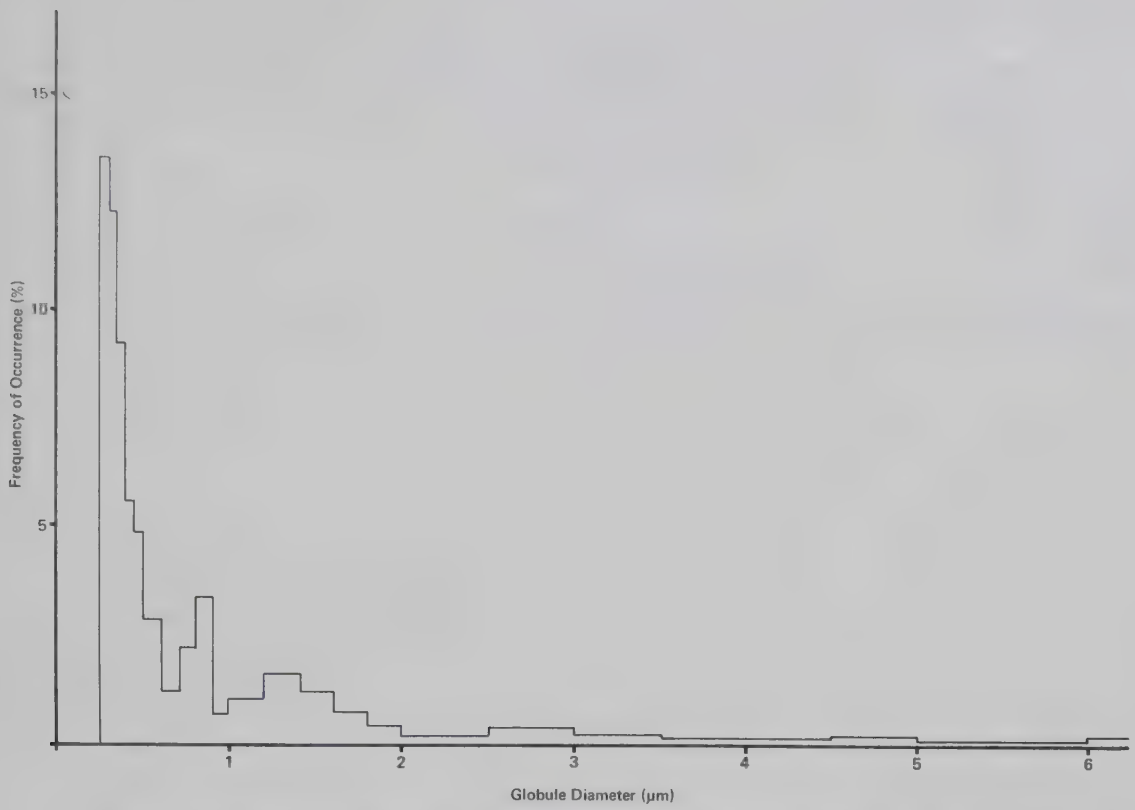


Fig. 5.—Globule size distribution. D. Manually prepared emulsion after homogenisation.

The results are shown in Figures 2-5. They indicate that colloid milling has the reserve effect to that predicted by the globule reduction hypothesis. It will be seen that the emulsion obtained from coconut kernel by manual operation involving minimum necessary force contained a high proportion of small globules —e.g. 63 per cent had a diameter below 1 m, even ignoring those below the limit of observation— and that this proportion was substantially less in emulsions prepared by colloid milling. In view of the limited amount of data available at present this conclusion must be regarded as tentative; further centrifugal sedimentation studies will be carried out.

It appears that the weighted mean diameter of globules in coconut emulsions is fat lower than has been supposed. The data recorded in Figures 2-5 indicate a value of 1.6 μm for manually prepared emulsion, reduced to 1.2 μm by homogenisation. The values for the emulsions prepared by colloid milling were respectively 3.6 μm and 3.9 μm. These measurements are consistent with the qualitative observation that emulsions derived from colloid milling cream far more rapidly than those prepared manually— rates in both instances being greater than the theoretical values calculated from Stokes' Law. It must be noted, however, that slight aeration of the emulsion occurs during colloid milling; no doubt this contributes to enhancement of creaming rates.

It is concluded that the colloid mill has definite advantages over the WCDP mill for use in the wet milling of coconut kernel, though both are capable of achieving the high efficiency of oil release required in a commercial process. A marked disadvantage of the colloid mill, however, is its exacting feedstock size requirements and its relatively low throughput capacity. This applies at all setting of the mill, and so would apply to all stages of a wet milling system involving sequential milling in a series of colloid mills. By comparison, the WCDP mill is characterised by high throughput capacity at settings which give oil extractions up to about 90 per cent. The most satisfactory milling system, therefore, is the one

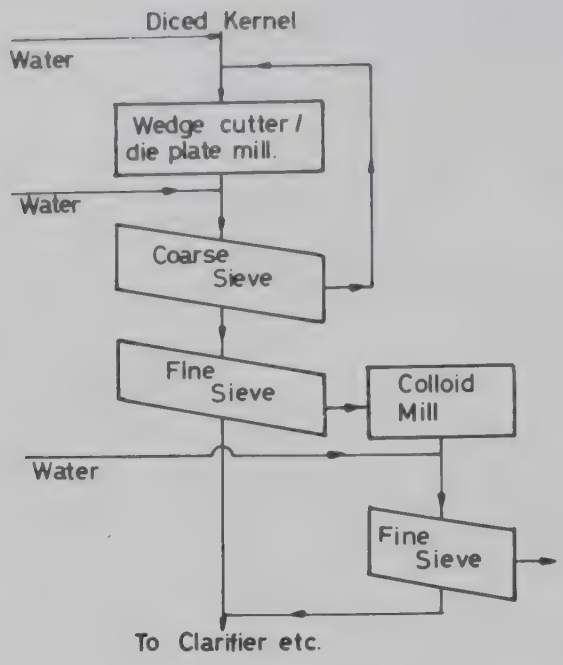


Figure 6.

illustrated in Figure 6 - a WCDP mill operating in tandem with a colloid mill, with feed back of part of the residue of the first milling operation. The variables in this system are: the size of the die plate perforations; the clearance between the cutter and the die plate; and the mesh size of the coarse and fine screens. Work is now in progress to determine the values of these variables for optimal throughput of the system for particular settings of the colloid mill.

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Large scale continuous production of soybean protein hydrolyzate in a constant flux membrane reactor

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ABSTRACT

The continuous conversion of a soybean protein isolate into soluble peptides by the thermostable acid protease from *Penicillium duponti* has been studied in a constant flux membrane reactor. A 94 % protein conversion was achieved in one hour reaction time, at pH 3.7 and 60°C, with a product output of up to 1 kg/hr per 10 ft^2 of membrane surface. The enzymatic reaction has been optimized, and the relevant kinetic and mechanistic aspects of the proteolysis in a constant flux membrane reactor are discussed. The concepts of capacity and productivity of an enzyme reactor are advanced, in order to contrast the characteristics of the continuous over the conventional batch process.

INTRODUCTION

Continuous enzymatic reactions conducted in membrane reactors have been proven feasible in several occasions (Blatt *et al.*, 1968; Wang *et al.*, 1970; Ghose and Kostick, 1970) and in theory offer advantages over the conventional batch reactions.

Batch enzymations are usually conducted with substrate to enzyme weight ratios of 100-300, conditions under which the initial rate depends solely on the enzyme concentration. However, the reaction shows a decrease of the reaction rate with time due to: a) decrease in the concentration of substrate; b) decrease in the concentration of active enzyme, either through inactivation and/or reversible inhibition by the reaction products. As a result, the output of a batch process, defined as the mass of product produced per unit time, decreases as the reaction proceeds. Batch reactions are usually terminated by heat inactivation of the remaining enzyme, once the rate of the chemical changes become negligible.

The present availability of ultrafiltration systems fitted with membranes of selected sieving ability, have made it possible to approach reaction conditions

having high, steady product outputs: a) by keeping the substrate and enzyme concentrations constant; b) by using large enzyme/substrate ratios, taking advantage of the enzyme retention capability of the membrane.

The enzyme reactors are particularly suitable for hydrolysis of biopolymers (proteins, starches, celluloses and nucleic acids), because membranes can be chosen to allow only the permeation of products.

As shown in this paper, the achievement of a fast reaction rate allows a short residence time for the conversion of the substrate into diffusible products. When operated at constant flux, these reactors can reach steady state quickly and are able to sustain quantitative conversions for extended periods of time at constant rates of production.

The preparation of protein hydrolyzates that could function as ingredients in the engineering of novel nutritional products faces severe restrictions, ranging from palatability to nutritional specifications. These requirements must be met in practice within the constraints imposed by the need of an ample supply of the protein substrate at a reasonable low cost. A sensible hydrolytic process that could convert any cheap, denatured protein into a water soluble peptide mixture is an important goal being sought by many laboratories. The mild conditions intrinsic to the use of proteases are desirable in terms of minimizing the formation of off-flavors, and preserving the chemically sensitive essential amino acids tryptophan, methionine, threonine and lysine.

The nature of enzyme is of importance, in terms of availability, cost, rate and specificity. The first two considerations leave little choice but the use of microbial enzymes. The last two are even more restrictive when selecting an endopeptidase whose specificity will allow the rapid production of soluble, mon-bitter peptides in high yield. And lastly, the practical requirements of processing impose pH-temperature conditions under which the chances of microbial spoilage are minimal.

This paper discusses the hydrolysis of a commercially available soybean protein isolate (PROMINE R, Central Soya Co., Chicago, I11.) with a fungal acid protease produced by the thermophilic *Penicillium duponti* K1014 (Hashimoto, *et al.*, 1973), made available to us by Kikkoman Shoyu Co., Noda, Japan. This enzyme can operate effectively at 60° C at pH 3.7-4.2 without appreciable loss of activity, and with a turnover rate as high as pepsin. Unlike pepsin (Fujimaki, *et al.*, 1970) this fungal endopeptidase is able to hydrolyze soybean protein without the formation of bitter peptides.

The present study has been done on an experimental ultrafiltration unit (Aqua-Chem Inc., Milwaukee, Wisc.) to test the feasibility of continuous enzymation under fully controlled conditions, and at a sizable, meaningful scale. The reactor, having an operative volume (V_r) of 40 1 was connected to a laminar flow module of 10 ft^2 of cellulose acetate membrane, with a porosity cut-off between 10,000 - 20,000 daltons. The reactor was operated keeping the following parameters constant: pH, temperature, flux rate and reaction volume.

METHODS AND MATERIALS

Ultrafiltration Unit.

The ultrafiltration unit used in the work was designed and constructed by Aqua-Chem, Inc. A schematic diagram of the unit is shown in Figure 1. A laminar flow module (1) containing 10 ft^2 of cellulose acetate membrane was used in conjunction with circulation rates of 5-20 gal/min and pressures

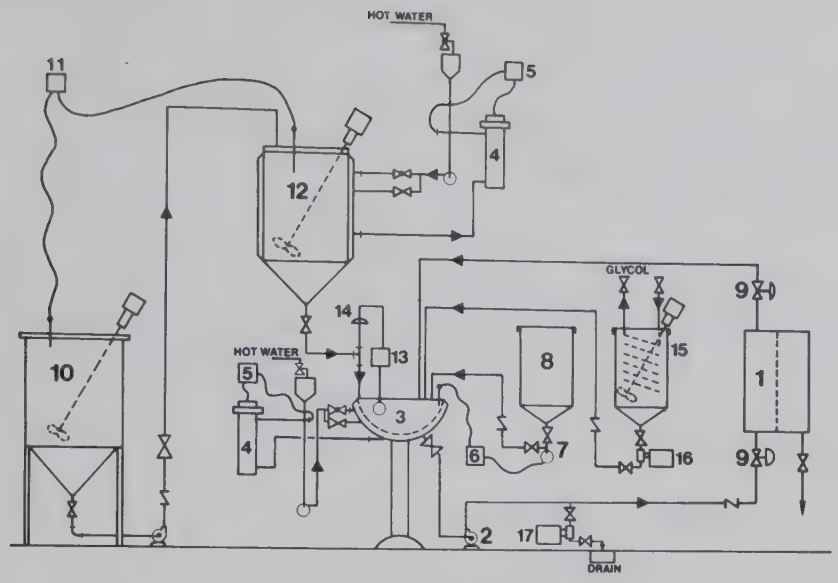


Fig. 1.–Ultrafiltration unit.

of 0-40 psig through the module supplied by the pump (2). The operating temperature was maintained at 60° C in a jacketed reaction tank (3) by means of a circulating hot water system, having an electric heater (4) controlled by the thermostat (5). The pH was maintained constant at 3.7 with a pH-stat (6) controlling the motor valve (7) that delivered concentrated (85 %) H_3PO_4 from the tank (8) as needed. Constant flux rate through the membranes, essential for efficient operation of the process, was maintained by adjusting the throttle valves (9), thus varying the circulation rate and pressure across the membranes to prevent the decrease in flux rate that otherwise would occur during the runs. The substrate feed, Promine R suspensions at 15 to 34 g/1 preadjusted to pH 3.7 with 85 % H_3PO_4, was prepared in tank (10) fitted with pH meter (11) in 80 l batches during the course of the runs, and kept with stirring in a jacketed holding tank (12) at 60°. The volume of the reaction was maintained constant at 37 ± 3 l by addition of the substrate suspension from the holding tank (12), and was regulated by the float level control (13) acting on the control valve (14). In order to maintain a constant level of enzyme in the reactor, enzyme was fed from the refrigerated tank (15) with a pump (16) at the rate needed to compensate for enzyme losses. When necessary, bleeding of the reactor was performed by operating the pump (17).

Analytical Methods.

The following quantities were measured during the course of the runs:

[S]: concentration of Promine R in the feed, in g/1, determined by dry weight at 110° for 24 hrs.

[P]: concentration of filtrable peptides in the ultrafiltrate, in g/1, measured by the biuret method (Gornall **et al.**, 1949) using a standard curve prepared with a sample of peptides previously made with *P. duponti* protease.

[R]: concentratión of total solids in the reactor, in g/1, determined in the same fashion as S.

[Q]: Concentration of inert material accumulated in the reactor, in g/1, calculated from the expression.

$$[Q]_t = \frac{0.0845\ S_t}{V_r}$$

The hydrolysis of Promine R with *P. duponti* acid protease produces from the substrate 8.45 % by weight of an insoluble, non-permeable material that is refractive to further digestion by the *P. duponti* protease (see Results and Discussion). The accumulation of Q in the retentate is a function of the substrate passed into the reactor as indicated in the above expression, where V_r is the reactor volume and S_t is the cummulative mass of substrate passed into the reactor at time *t*, calculated from the values of flux Φand [S].

[RS]: concentration of the reactor solids which can be converted into product P, in g/l. Separate ultrafiltration experiments done on retentate aliquots, using samples of fresh membrane identical to the one used in the large unit, have shown that the concentration of filtrable

peptides, inside the reactor is equal to [P]. Therefore, [RS] was calculated as:

$$[RS] = [R] - ([P] + [Q]_t)$$

Enzyme concentrations were determined by assay at pH 2.50 and 30° C against casein as described previously (Emi *et al.*, 1974) and are expressed as g/l of pure *P. duponti* acid protease preparation, having a specific activity of 4800 PU/mg. Crude *P. duponti* acid protease, having specific activity of 640 PU/mg was used throughout all of this work and also served as stardard in every assay.

$[E_R]$: assayable enzyme in the reactor, g/l.

$[e_F]$: concentration of enzyme in ultrafiltrate, g/l.

Instantaneous yield of the reaction, ϵ, was calculated from the ratio of the feed stream and the permeate stream, at any time: $\epsilon = [P]/[S]$.

Flux, Φ, 1/hr, was determined by a graduated cylinder and stopwatch.

Reaction time, in hr, is the time required to replace the volume in the reactor (V_r) with an equal volume of feed and is equal to V_r/Φ

Reactor output, in g/hr, is equal to $[P]\Phi$.

Velocity of the reaction, in g/l. hr, is equal to $\frac{[P]\,\Phi}{V_r}$.

RESULTS AND DISCUSSION

The conversion yield (ϵ) was studied as a function of the initial substrate/enzyme weight ratio (S/E) and flux (Φ). The realtionship between these variables is given by the following expression for the **capacity** K of the reactor, defined as the mass of peptides produced per unit mass of enzyme per unit time.

$$K = \frac{[S]}{[E]} \cdot \epsilon \cdot \frac{\Phi}{V_r} = \frac{[P]}{[E]} \cdot \frac{\Phi}{V_r} \qquad (1)$$

The factor (Φ/V_r) represents the number of reaction volume passed through the reactor per unit time. The dimension of capacity is reciprocal time, and its value approximates the first order rate constant of the overall reaction: Substrate (S)→ Permeable peptides (P). Consequently, K is the reaction velocity, $[P]\Phi/V_r$, divided by the enzyme concentration [E] in the reactor, as shown in equation *1*. The factors $[S]/[E]$, ϵ and Φ/V_r are interdependent and their product is constant K. The value of K is a measure of the reactor output at a given pH and temperature.

Optimization of the Reactor.

The reactor was optimized by determining first what was the limiting value of $\frac{[S]}{[E]}$ corresponding to a conversion yield $\epsilon \geq 0.90$. In order to maximize K (equation *1*), $(\frac{[S]}{[E]_{max}})$ was sought experimentally for [S] = 15 g/l by determining the minimal [E] needed to achieve the above efficiency. It was found that a $\frac{[S]}{[E]}$ of 26 was necessary in order to achieve $\epsilon = 0.94$ on a protein basis. The [S]/[E] values are expressed on a weight basis with respect to pure enzyme, and represent the values at which the reaction was started. The optimal value of $\frac{\Phi}{V_r}$ is determined by the maximal flux consistent with a value for ϵ equal to 0.94. If a flux above the optimum value is used, the rate of substrate introduction will exceed the rate of product formation, resulting in a decrease in ϵ and a concomitant build up of solids in the reactor. For the unit under consideration (10 ft^2 membrane) and for the reaction parameters adopted (60°, pH 3.7), it was found that the maximum flux

allowable was 40 l/hr, corresponding to $\frac{\Phi}{V_r} = 1\ hr^{-1}$, or a 1 hr residence time for the substrate in the reactor. The effect of initial enzyme concentration on the yield and reaction velocity $\frac{\Phi[P]}{V_r}$ is seen in Figure 2, for two runs at $\Phi/V_r = 1$ hr at 15 g/l substrate feed and having initial [S]/[E] ratios of 26 and 62. Evidently at the lower enzyme concentration ([S]/[E]) = 62), the rate of product formation is insufficient to allow quantitative conversion of substrate to product throughout the run. At the [S]/[E] ratio of 26, product formation proceeds at a rate sufficient to result in total conversion, indicating that a large amount of enzyme is essential to obtain a quantitative yield in one hour reaction time. A similar dependence is depicted in Figure 3, recording the relationship between ϵ and $\frac{[RS]}{[E^*]}$ ratios, measured during the course of different runs; values of $\epsilon > 0.90$ were found only when $\frac{[RS]}{[E^*]} \leqslant 60$.

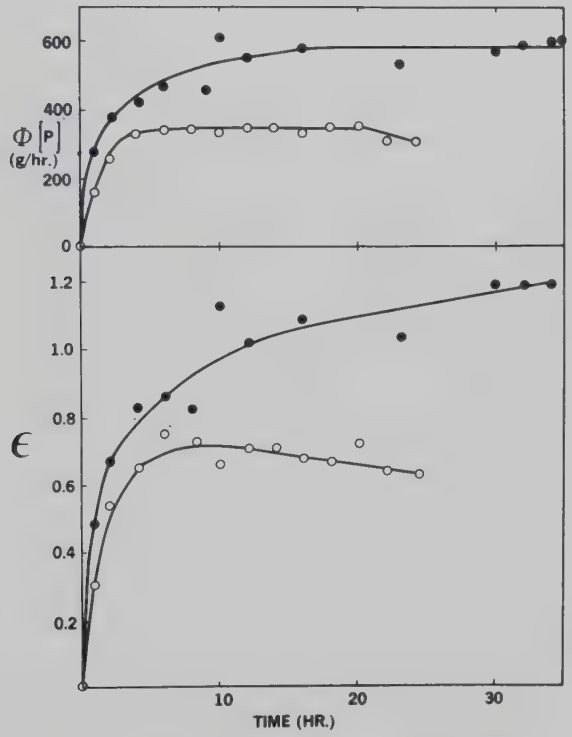


Fig. 2.–Effect of initial enzyme concentration on the product output and yield of the reaction, at 15 g/l feed.
●—●, [S/E] = 26; ○—○, [S/E] = 62

Effective Enzyme Concentration [E*] in the Reactor. Significance for Optimal Enzyme Management.

In order to maintain the reaction rate constant, enzyme had to be replaced at a rate of 2 % of the enzyme in the reactor per hr in order to compensate for the enzyme loss by permeation through the membrane, and by thermal inactivation. A careful measurement of the thermostability of the *P. duponti* protease preparation, in the presence of substrate, at 60° and pH 3.7, showed that the enzyme is quite

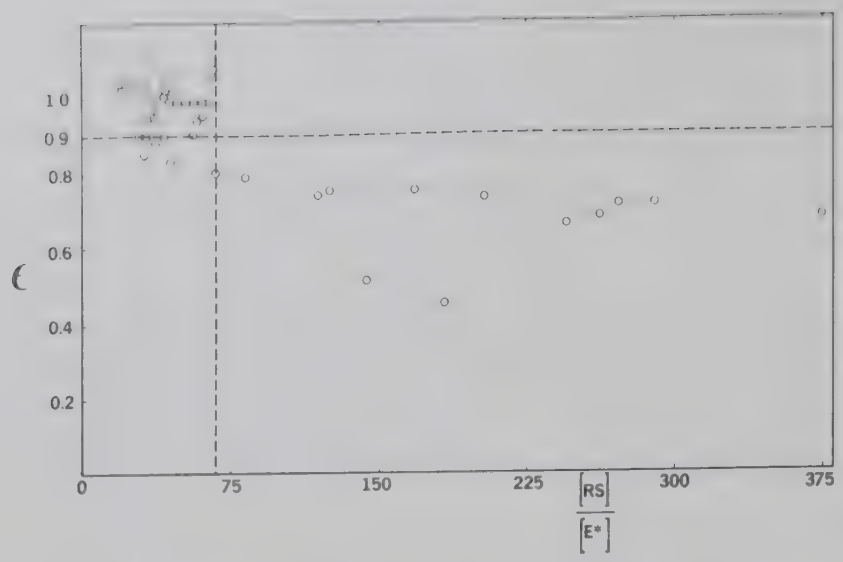


Fig. 3.–Dependence of yield ϵ upon the substrate to enzyme ratios found in the reactor at different times, expressed as the available substrate in the reactor RS over the accounted enzyme E*.

stable and decayed at a rate no greater than 0.2-0.5 %/hr, measured over a period of 10 hr.

After performing periodic enzyme assays on the retentate and permeate streams, we observed in all runs that the accounted enzyme concentration in the reactor [E*], calculated from the initial enzyme concentration by computing the known addition and losses experienced up to time t, was always higher than the enzyme concentration $[E_R]$ directly assayable in the retentate at this time. The difference $\Delta[E] = [E^*] - [E_R]$ varied with time during a run and amounted to an average disappearance rate of 2 %/hr based on the activity present in the reactor.

Good evidence that the apparently missing enzyme $\Delta[E]$ remains active comes from kinetic considerations. Given the expression for capacity (equation *1*), it was observed that only the accounted value or effective enzyme concentration [E*] gave a constant value of capacity for a reactor at the steady state. An example of this is shown in Figure 4, for a run done at 15 g/l feed. In other words, the rate of product formation is strictly proportional to [E*] but not to $[E_R]$, suggesting that the difference $\Delta[E]$ represents a form of active enzyme removed from circulation, for example, impacted in the ultrafiltration module.

Independent evidence of the participation of $\Delta[E^*]$ in protein hydrolysis comes from the examination of the time course changes of [RS], the concentration of available substrate in the reactor, in relation to changes in enzyme concentration. Figures 5 A-C reproduce data of three runs at 15 g/l feed, showing that [RS] is more dependent upon changes in [E*] than that of the assayable $[E_R]$. Figure 5 A denotes a run in which maintenance of a steady state with quantitative yield of protein to filtrable peptides was achieved. This case is a good example of efficient use of the enzyme. On the other hand, Figure 5 B illustrates a run in which too much enzyme is present. Although $[E_R]$ remained constant throughout at 0.60 g/l. [E*] increased from 0.67 to 1.64 g/l, resulting in a decrease in [RS] and loss of the steady state. In contrast to Figures 5 A and 5 B, Figure 5 C illustrates the case of a reaction that failed to reach steady state because of the insufficient initial enzyme concentration. In consequence [RS] rose sharply, and $[E_R]$ was observed to decrease noticeably over the short period of 24 hr, while [E*] remained constant. Because 80-95 % of RS is known to be insoluble under the conditions of the reaction, then the observed $\Delta[E]$ can be interpreted in terms of enzyme association

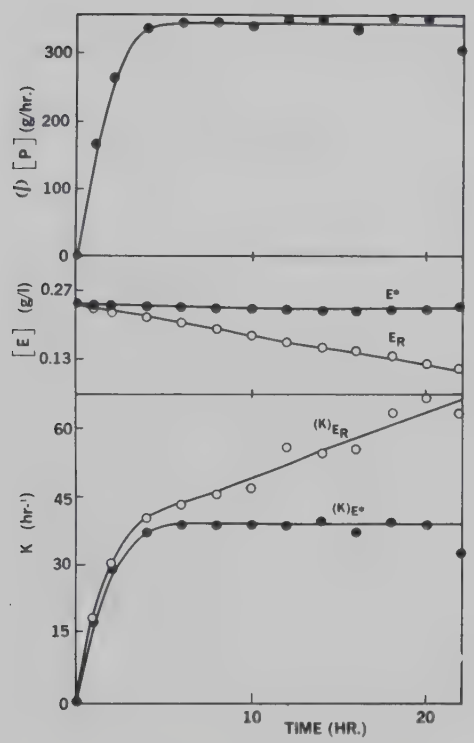


Fig. 4.–Relationship between product output and enzyme concentration in the reactor, $(K)_{E^*}$ is the capacity calculated for $[E^*]$, and $(K)_{E_R}$ the value found based on $[E_R]$.

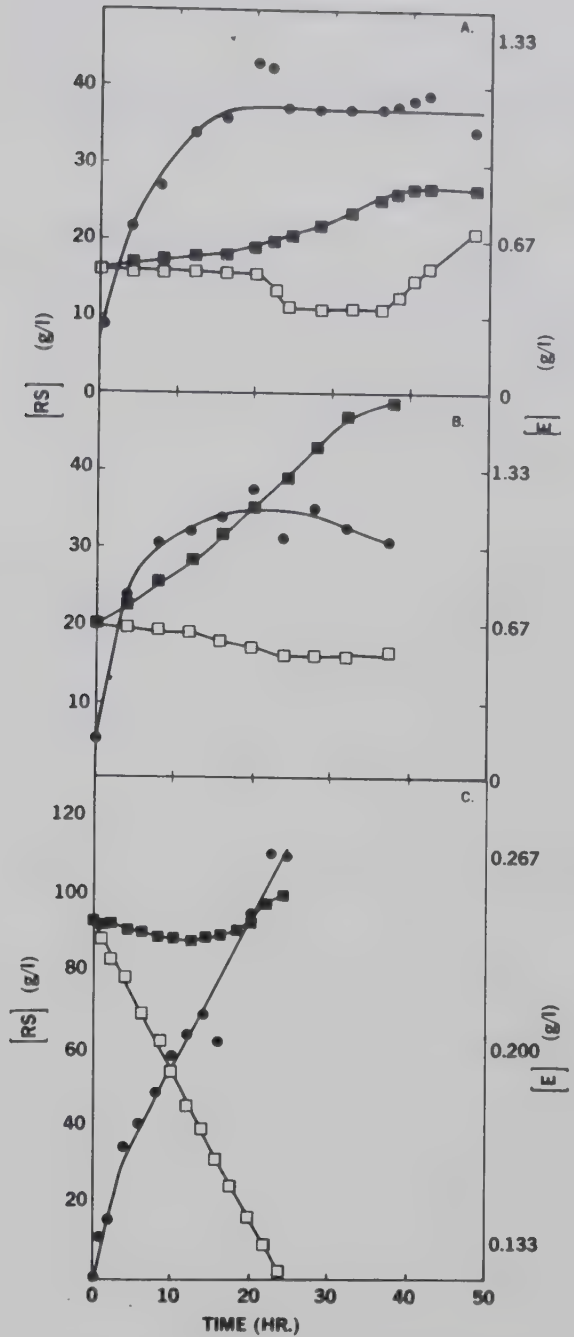


Fig. 5.–Dependence of the level of available substrate in the reactor upon enzyme concentration.
●——●, available substrate RS; ■——■, $[E^*]$; □——□, $[E_R]$.

to insoluble substrate and impactation of the complex in the ultrafiltration module, an effect that will become more significant at very high [RS].

In order to explain a steady state equilibrium characterized by a constant value of K (Figure 4) and constant level of available substrate [RS] in the reactor (Figure 5 A), it is necessary to postulate that Δ[E] is active and participates in the maintenance of the stasis.

Control of Retentate Concentration by Bleeding.

Shown in Table la is an analysis of the feed substrate, Promine R, and Q, the

TABLE 1

Soybean Protein Substrate and Q, the Refractile Residue of Continuous Proteolysis

	Substrate	Q
a) *Chemical Composition (dry weight basis)*		
% Protein (N X 6.25)	90.9	67.3
% Carbohydrate (as glucose)	1.18	2.86
% Lipid extracted by C_2H_5OH/H_2O (8/2, v/v)	2.6	27.0
% Lipid extracted by C_2H_5OH/H_2O (8/2, v/v) followed by $CHCl_3/CH_3OH$ (2/1, v/v)	1.5	4.0
% Lipid, total	4.1	31.0
b) *% Q in Substrate*		
Wt. Substrate passed through reactor	19.162 kg	
Wt. Q isolated from reactor after exhaustive digestion	1.619 kg	
% Q in substrate	8.45	
% Permeable material in substrate	91.55	
c) *Grams of Substrate Components Retained in Q per 100 g of Substrate Processed*		
Protein	5.42 g	
Carbohydrate	0.24 g	
Lipid	2.59 g	
	8.45 g	
d) *Nonpermeable Components, Expressed as % of Total Components Fed into Reactor*		
Protein	6.2	
Carbohydrate	20.0	
Lipid	64.7	

inert material isolated at the end of a prolonged continuous run. Q is the nonpermeable, insoluble residue derived from Promine R and has been shown to be totally refractive to further digestion by *P. duponti* acid protease, several other acid endopeptidases, and thermolysin, a neutral endopeptidase. To date, only exopeptidase-containing enzymes, such as Pronase and *Bacillus subtilis* aminopeptidase, can digest Q. Further work has shown that Q is an aggregate of peptides enriched in hydrophobic amino acids, particularly valine and leucine, nucleated around hydrophobic lipoid impurities, known to be associated with the substrate.

Table 1b indicates that Q comprises 8.45 % by weight of the protein substrate and that the bulk of Q is proteinaceous. Using *P. duponti* acid protease in the continuous system, we can thereby convert 93.8 % of the protein to filtrable peptides. Even though the substrate contains 4.1 % of lipoidal material, mainly triglycerides, phospholipids, saponins, and sitosterol (Nash *et al.*, 1967) 65 % of these lipids are nonpermeable and accumulate in the reactor as indicated by Table 1d. Thus, peptide aggregation around lipoidal impurities results in an unavoidable build-up, equivalent to an increase of [Q] of 22.8 g/l for every 10 kg of substrate processed.

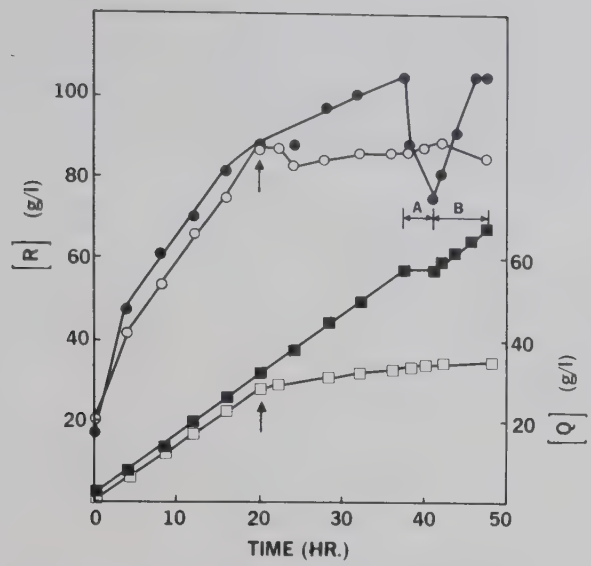


Fig. 6.–Control of [Q] and [R] by bleeding. Open symbols correspond to a reaction bled starting at 20 hrs (arrows): ○—○, [R]; □—□, [Q]. Closed symbols indicate the course of a reaction with no bleeding, subjected to a water wash of duration A, followed by reassumption of substrate feed B: ●—●, [R]; ■—■, [Q].

Figure 6 illustrates the effect of a 3 % Vr/hr bleeding on a reaction having 15 g/l substrate feed, in relation to a reaction that was not bled. In the former the total solids concentration in the reactor, [R], was maintained at 85 g/l, once the bleeding was established at 20 hrs into the run, resulting from the control over the build up of the inert material Q. As an alternative way of bleeding, the reduction of the reactor solids was attempted by replacing the substrate feed with water, as indicated in Figure 6. However, water washing was less effective because: 1) no protein was fed, thus reducing the rate of product formation; 2) no inert material Q was removed from the reactor; 3) the [R] rose rapidly upon reintroduction of substrate. Therefore, water washing is considered inferior to bleeding as a means to control the build up of solids in the reactor.

Enzyme Losses: Concept of Productivity

In order to maintain the reaction rate constant, it is necessary to replace the amount of enzyme being lost by permeation through the membrane. thermal inactivation, and bleeding.

If this enzyme replacement rate is considered in the equation *1* for capacity instead of the total enzyme present, an alternative concept, productivity (Π), is defined for the continuous reactor, as the mass of product produced per unit mass of enzyme replaced per unit time. Productivity is thus an operative concept that defines properly the enzyme usage of a continuous reactor.

In Table 2, values of this parameter along with other significant data are given for runs at 15 g/l substrate feed with and without bleeding to control the reactor concentration. Based on data of the previous sections, ΔE is considered active; therefore, the only significant losses of enzyme should be permeation and, when applicable, bleeding.

Productivity is markedly reduced by bleeding, whereas the product output and capacity are unaffected. The data in Table 2 define the observed limits of variation in Π. Bleeding is essential for the continuous operation of the process; therefore, the

TABLE 2

Capacity and Productivity of the Membrane Reactor Hydrolysis of Soybean Protein with P. duponti *Acid Protease at pH 3.7 and 60° C, and [S]/[E] of 26*

Parameter	Run No. #1	#2	#3	#4	Average values
Substrate feed, [S] (g/l).	15	15	15	17	
Length of run (hr).	48	70	36	37.5	
Overall conversion yield, ϵ (weight basis).	0.91	0.85	0.90	0.95	0.90
$\frac{\Phi}{V_r}$, (hr^{-1}).	0.98	0.99	0.97	0.97	0.98
Bleed (% Vr/hr).	3	3	0	0	
Enzyme loss (g/hr) By permeation.	0.4	0.6	0.4	0.2	0.4
By bleeding.	0.5	0.3	0	0	0.4
Total.	0.9	0.9	0.4	0.2	
Reactor output, Φ[P], (g/hr).	527	467	573	597	541
Capacity, K, (hr^{-1}).	18.6	28.2	26.6	24.4	24.4
Productivity, Π, (hr^{-1}).	567	514	1293	2411	

high values of Π for the unbled reactions are not realistic in terms of a practical process. On the other hand, the lower values of Π in the bled reactions are not representative of an efficiently conducted process. In practice one would recover the enzyme from the bled stream by centrifugation and return the solubles to the reactor. The variation of the productivity as a function of the amount of bled enzyme discarded is shown in Table 3; II has been calculated from the average experimental values found for [P]$\Phi$and enzyme losses. The Π value of 966 hr^{-1} corresponding to a 60 % recovery of bled enzyme is considered realistic in terms of an industrial process.

TABLE 3

Effect of the Recovery of Bled Enzyme upon Reactor Productivity

% Bled Enzyme Recovered	Productivity (hr^{-1})
0.	675
20.	751
40.	845
60.	966
80.	1127
100.	1350

Continuous proteolysis at 15 g/l feed of soybean protein with *P. duponti* acid protease, at 60° C and pH 3.7.

Calculation based on the data of Table 2, adopting an average product output of Φ [P] = 541 g/hr, and average enzyme losses of 0.4 g/hr by permeation and 0.4 g/hr by bleeding.

Steady State Kinetics of the Membrane Reactor.

The course of proteolysis is a complex, multistep, sequential process, during which a multitude of intermediates are generated and degraded, until all the bonds

susceptible to the enzyme are split. The kinetics results from a sequence of transient Michaelis-Menten equilibria of varying K_m and rate constant k values. Therefore, a single rate constant cannot be derived for describing the kinetics of a batchwise proteolysis carried to completion.

When proteolysis occurs within a membrane reactor, however, the membrane classifies the intermediates on a permeability basis into two populations, RS and P, so defining the chemical reaction,

$$RS \quad \text{(nonpermeable)} \xrightarrow{\bar{k}} P \quad \text{(permeable)},$$

$\bar{k}$ being the rate constant of the slowest steps in the RS population which produce permeable product.

Following this approach, we have evaluated the kinetics of the continuous proteolysis. In Figure 7 are reproduced the pertinent experimental values obtained under steady state conditions. Km was determined for the initial hydrolysis of RS by *P. duponti* protease at 60°, pH 3.7, and gave a value of 27 g/l. The value of k was derived from the Michaelis-Menten equation

$$\bar{k} = \bar{v} \cdot \frac{K_m + [RS]}{[E] \cdot [RS]} = 44.0\ hr^{-1} \qquad (2)$$

corresponding to the equilibrium $RS + E \rightleftarrows E{\cdot}RS \rightarrow P + E$. This average rate constant is considerably smaller than the rate constant measured for the initial hydrolysis os soybean protein, at the same pH and temperature, which was $k_o = 3015\ hr^{-1}$.

Comparison of Capacity and Productivity of Continuous and Batch Proteolysis.

Capacity, defined as grams of peptides produced per gram of enzyme present per hour, is an operational concept useful to compare overall outputs for both processes. For the continuous process, capacity is given by equation (3), and in theory equals the rate constant $\bar{k}$ if $K_m = 0$.

$$K_{continuous} = \frac{\bar{V}}{[E]} = 24\ hr^{-1} \qquad (3)$$

Unlike the batch process, $K_{continuous}$ is independent of time, because both the velocity and enzyme concentration are kept constant in the reactor under steady state.

The batch reaction proceeds with a velocity that decreases exponentially with time, due to the continually decreasing substrate concentration. To determine the capacity of a batch reactor, the optimal conditions for the batch digestion of soybean protein with the acid protease of *Penicillium duponti* at pH 3.7 and 60° C were determined, in terms of the highest $\frac{[S]}{[E]}$ ratio able to yield $\epsilon = 0.94$ in the shortest time. It was found that for a reaction run with $[S] = 50$ g/l and $[E] = 0.25$ g/l ($\frac{[S]}{[E]} = 200$) 20 hrs were needed to achieve 94 % conversion yield (on protein basis). Assuming no enzyme destruction during such period, the batch capacity is:

$$K_{batch} = \frac{P}{E\,x\,t} = \frac{47}{0.25 \times 20} = 9.4\ hr^{-1} \qquad (4)$$

The comparison of capacities then indicates that on equal reaction volume basis, the continuous process will yield 2.6 times more peptides per unit time than the batch under comparable conditions. This effect is soledy due to the kinetic differences between both systems, as illustrated in Figure 8. The straight line denotes the rate of product formation in the continuous reactor at the steady state (Figure 7), operating at a velocity $\overline{v} = 13.6 \frac{g}{\cdot 1 \cdot hr}$. The hypothetical curve approximates the rate of product formation when the steady state of the continuous process is disrupted by stopping the substrate feed thereby forcing the reaction to proceed batchwise. The curve has been calculated from the integrated form of the michaelis-Menten equation.

$$[P] = [S_o] - [S_o] \cdot e^{-k'[E]t} \qquad (5)$$

adopting the kinetic parameters of the steady state ($\overline{k} = 44\ hr^{-1}$ $K_m = 27$ g/l; $k' = \frac{\overline{k}}{K_m} = 1.63$; $[S_o] = 32$ g/l). This expression predicts that it will take 7 hrs to

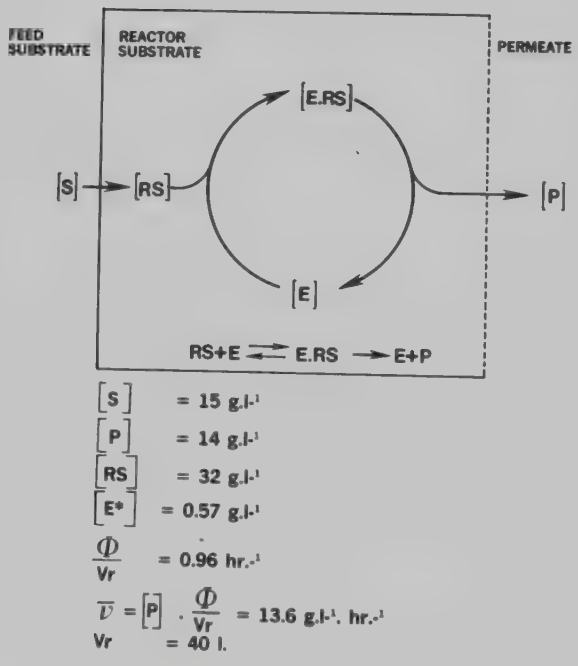


Fig. 7.–Scheme illustrating the continuous conversion of soybean protein into diffusible peptides by *Penicillium duponti* acid protease. The relevant parameters for the steady state are listed below the scheme, for a reaction conducted at pH 3.7 and 60°C, with 15 g/l feed and initial [S/E] of 26.

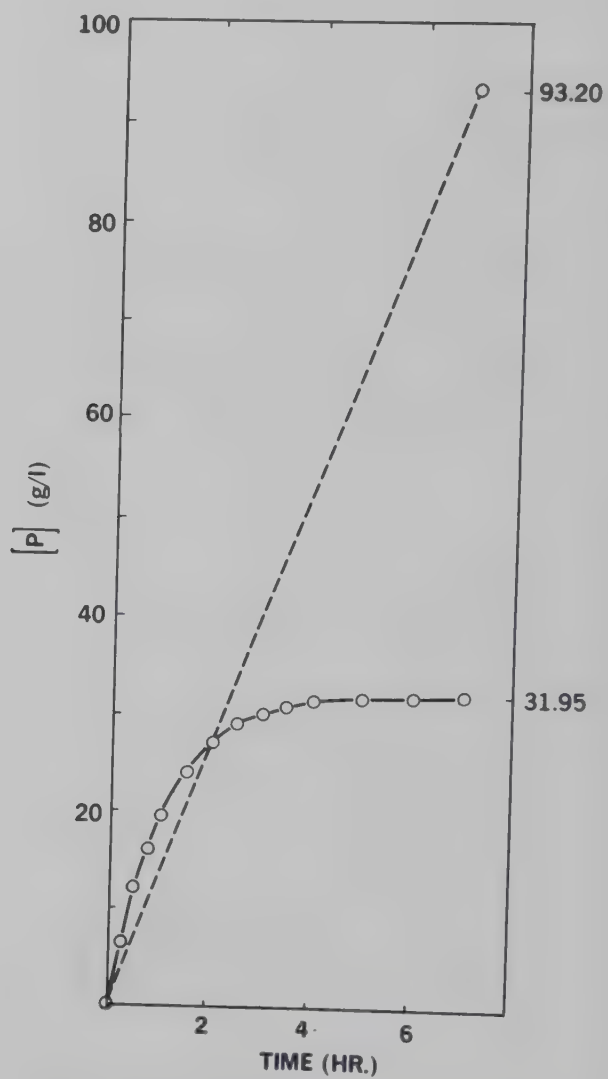


Fig. 8.–Differences in rate of product formation, illustrated for continuous and batch enzymations: (- - -), continuous reactions; (—) calculated for a batch reaction.

convert all the substrate into product, with an output of 31.95 grams per liter of reaction volume. The corresponding ouptut after 7 hrs of continuous reaction is 93.2 g/l or a ratio $\frac{\text{Output continuous}}{\text{Output batch}} = 2.9$, a predicted value in good agreement with the experimental ratio of 2.6 found for the capacities.

Besides the gain in rate, the continuous operation offers an advantage in enzyme usage over that of the batch. In the latter, the proteolysis is usually terminated by thermal inactivation of the enzyme, thus giving a productivity value identical to the capacity, or 9.4 g of product, produced per g of enzyme used per hour, in the above batch experiment.

On the other hand, a continuous reactor operating at steady state at 15 g/l feed was shown to have a productivity of 966 hr^{-1} (Table 3), when 60 % of the enzyme removed with the bleed was recycled. Therefore, the productivity of the continuous process is 100 times higher than that of the batch, despite the 10-fold increase in enzyme concentration used in the reactor.

Preliminary experiments at 30 g/l feed, keeping constant the ratio $\frac{[S]}{[E]}$ of 26, have resulted in a 2-fold increase in product output, up to 1 kg/hr per 10 ft^2 of membrane. However, the capabilities of the present ultrafiltration unit were exceeded, mainly in terms of circulation pump capacity and module design.

Further improvements of this continuous process for protein hydrolysis are foreseable, and will result from progress in the areas of membrane technology and enzyme immobilization research.

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Chemical and nutritional evaluation of enzymatic soybean hydrolyzates

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ABSTRACT

Hydrolyzates have been prepared by digesting a commercial soybean protein isolate with the fungal acid protease from *Penicillium duponti* at pH 3.7 and 60°C, under conditions of continuous and batch enzymation. Both types of hydrolyzate have been characterized in terms of amino acid composition, chemical estimation of average molecular weight, and molecular weight distribution following permeation through Biogel P-4 and were found similar. The protein quality of the continuous hydrolyzate was evaluated using the PER rat assay. The values obtained, relative to casein PER = 2.50, were 1.37 as is, and 2.02 after 1 % methionine addition on a protein basis. The corresponding values for the soybean protein substrate were 1.33 and 2.23. The solubility and palatability of these soybean protein hydrolyzates are advantageous attributes for the protein fortification of food products.

INTRODUCTION

As a by-product of edible oil production, oilseed proteins are a potentially important source of human dietary protein throughout the world (Milner, 1966). Following oil removal the protein present in the defatted cakes is heat-denatured and therefore directly unextractable. Hence proteolysis is an attrative approach for recovering the protein from oilseed press cakes in soluble form and affords a high protein preparation suitable for protein fortification of a wide variety of foods. As a first step towards this goal, we have investigated the preparation and properties of enzymatic hydrolyzates of a commercial soybean protein isolate. Choice of a suitable enzyme was primatily based on: (1) palatability of hydrolyzate, (2) yield of hydrolyzate, (3) processing conditions minimizing the possibility of microbial contamination. On the basis of these criteria, the thermostable fungal acid protease from *Penicillium duponti* (Hashimoto *et al.*, 1973) was selected.

This paper complements the study on the preparation of soyprotein hydrolyzate by the above enzyme using a membrane reactor in a continuous fashion

(Iacobucci, G. A. *et al.*, 1974). Peptides from the continuous process, operating at the steady state, were thoroughly characterized. In addition, peptides prepared by a batch process were characterized for comparison. Comparative PER studies have been done on the soyprotein isolate and the hydrolyzate derived by the continuous process to show that predigestion of the protein with this non-physiological protease did not impair amino acid utilization by the growing rat.

EXPERIMENTAL

A crude preparation of the *P. duponti* acid protease (640 proteolytic units/mg; Iacobucci *et al.*, 1974), kindly donated by the Kikkoman Shoyu Co., was used to prepare both the continuous and batch hydrolyzate from *Promine R*, a soyprotein isolate at the isoelectric state, obtained from Central Soya Co., Chicago, Ill.

Preparation of Hydrolyzates

Preparation of hydrolyzate by the continuous process has been described (Iacobucci **et al.**, 1974). Hydrolyzate was prepared batchwise by treatment of a 5 % *Promine R* suspension at pH 3.7 at 60° C for 21 hrs using 5800 proteolytic units/g *Promine R*. Phosphoric acid (3.7 M) was used to maintain the pH constant throughout the hydrolysis. Insolubles were removed by centrifugation prior to further processing.

Refinement and Isolation of Hydrolyzates

Hydrolyzates from both the batch and continuous process were similarly treated in the following manner at pH 3.7. Inactivation of enzyme was performed by holding the hydrolyzate solutions at 97° for 5 min. followed by rapid cooling to 30°. The resulting solutions were stirred with Darco G-60 charcoal (13.3 parts/100 parts by weight hydrolyzate solids) for 30 min. After being filtered through celite, the hydrolyzates were stirred at 25° for 30 min. with polyvinylpyrrolidone (Polyclar AT, obtained from GAF Corp.) using 1.7 parts/100 parts by weight of hydrolyzate, and refiltered through fresh celite. The deflavored hydrolyzates were concentrated to 30 % solids by wt. at 40-50° in vacuum and spray-dried with a centrifugal atomizing nozzle at an inlet temperature of 135°.

Hydrolyzate Characterization

Average chain length, n, was determined as the ratio of α-amino nitrogen after hydrolysis of the peptides to that the unhydrolyzed peptides. The ninhydrin reaction (Moore, 1968) was used to determine α-amino nitrogen, and the alkaline hydrolysis procedure of Hirs *et al.* (1956) was used to hydrolyze the peptides to free amino acids. *Average molecular weight,* $\overline{MW}_n$, was calculated as n x 120, where 120 is an adopted value of average residue molecular weight. *Gel permeation* was performed in 0.2 N acetic acid at 25° using Biogel P-4, P-6, P-10 obtained from Calbiochem and Sephadex G-15 from Pharmacia, Inc. Hydrolyzates (250-350 mg) in a 3 ml volume were applied to 28 x 1.5 cm columns of the various gels and permeation performed at flow rates of 12-6 ml/hr. Elution of material was followed by absorbance at 280 nm, by the biuret reaction (Gornall *et al,* 1949) and by the methods previously described to measure the average chain lenght and molecular weight. The latter allowed estimation of the molecular weight distribution of individual samples, as well as a comparison between the batch and continuous hydrolyzates. Permeation patterns have been expressed in terms of the ratio of volume eluted to void volume (V_e/V_o). Void volumes were determined using blue dextran (Pharmacia, Inc.).

PER determinations were performed by the Wisconsin Alumni Research Foundation (WARF) using the standard A. O. A. C. method (Horwitz, 1970).

Amino acid analyses were performed on a Beckman Model 120 C Amino Acid Analyzer equipped with a Beckman Model 125 Integrator. Methionine and half-cysteine were determined following performic acid oxidation (Moore, 1963) and tryptophan was determined after hydrolysis for 18 hr. at 120° in 5N NaOH (Wilkinson et al., 1976).

RESULTS AND DISCUSSION

The parameters of both the batch and continuous enzymations are shown in Table 1, and indicate that the latter process is more efficient. Both the yield and productivity (amount of hydrolyzate produced/enzyme unit destroyed/unit processing time) are significantly higher for the continuous than the batch process. On a dry weight basis, the hydrolyzate prepared by the continuous process contained 13.3 % N and 4.08 % P; values corresponding to 83 % protein (N x 6.25) and 13 % phosphate. The phosphate content found is equal to that calculated to be present as counterions to the charged protonated groups in the peptides at pH 3.7. Thus, 96 % by weight of the hydrolyzate is comprised of phosphate salts of peptides.

TABLE 1

Parameters of Processes for Hydrolyzate Preparation

Parameter	Process	
	Continuous	Batch
pH.	3.7	3.7
Temperature.	60°	60°
Substrate Concentration.	3 %	5 %
g Substrate/g Enz[a].	26	825
Yield (Protein Basis).	94 %	72 %
Enzymation Time.	1 hr.	21 hrs.
Productivity.	1144 hr^{-1}	28 hr^{-1}

[a] Expressed in terms of pure enzyme (4800 proteolytic units/mg): see Experimental section.

The essential amino acid content of both hydrolyzates is given in Table 2 along with that of the isolate from wich they were prepared. The content of essential amino acids in both hydrolyzates is slightly lower than that of the isolate. However, these decreases in the case of the hydrolyzate prepared by the continuous process are insufficient to cause a PER reduction with respect to the isolate as shown in Table 3. Methionine supplementation at a 1 % level on a protein basis increased the PER value of both preparations above 2 as compared to 1.35 with no supplementation. The results indicate that predigestion of the protein with *P. duponti* acid protease did not impair amino acid utilization by the growing rat.

The molecular weight range of the peptides prepared by the continuous process was estimated by gel permeation as shown in Fig. 1. The biuret permeation pattern obtained through Biogel P-4 (4000 MW exclusion limit) indicated that the range lay below 4000 daltons. The corresponding pattern through Sephadex G G-15 (1500 MW exclusion limit) indicates that a small portion of the peptides have a MW $\geqslant$ 1500 daltons, but that the bulk is below this value. The G-15 pattern also reveals that significant quantities of non-proteinaceous 280 nm absorbing material are present; their presence is seen at V_e / V_o values well beyond the tyrosine marker (Tyr in Fig. 1). Although less sensitive, the biuret reaction is a more specific measure of peptides than UV absorbance in hydrolyzates prepared from food-grade proteins since the latter usually contain significant amounts of UV absorbing material.

Molecular weight estimation by physical methods such as gel permeation will give falsely high values if aggregation occurs. To exclude this possibility and to

TABLE 2

Essential Amino Acid Content of Hydrolyzates (g aa/16g N)

	Isolate Promine R	Hydrolyzate	
		Continuous	Batch
Lysine	6.3	5.7	6.5
Threonine	3.9	3.3	3.0
Valine	5.3	4.4	4.1
Isoleucine	5.4	4.2	4.2
Leucine	8.9	7.1	7.2
Tyrosine	4.1	3.4	3.6
Phenylalanine	5.4	4.8	5.3
Half-Cystine[a]	1.2	1.1	0.8
Methionine[a]	1.3	1.0	1.1
Tryptophan[a]	1.2	0.9	0.8

[a] Determined by special procedures described in the Experimental Section.

TABLE 3

PER Values of Isolate and Hydrolyzate Prepared by Continuous Process

Sample	PER	
	As is	Corrected
Promine R	1.66	1.33
Hydrolyzate	1.71	1.37
Plus DL-Methionine[a]		
Promine R	2.78	2.23
Hydrolyzate	2.52	2.02
ANRC Casein	3.12	2.50

[a] Added at a level of 1 % on a protein basis.

provide an independent measure, we therefore determined by chemical means the molecular weight range of the hydrolyzates from the batch and continuous processes. The analytical method used for this purpose was shown to be valid by using a series of pure di- to hexapeptides; values obtained were within ± 10 % of theory. Average chain length and molecular weight were measured on the sample directly as well as on samples fractionated by gel permeation through Biogel P-4, P-6, and P-10 (corresponding exclusion limits of 4000, 6000 and 20.000 daltons). These measurements enabled the distributions of the mole and weight fractions *vs* molecular weight to be determined and compared for the peptides components of both hydrolyzates. When analyzed directly, the average chain length (n) and molecular weight ($\overline{MW}_n$) of the hydrolyzates were found similar: n was 3.1 (continuous) and 4.3 (batch), corresponding to $\overline{MW}_n$ of 370 and 520, respectively. Thus, *P. duponti* acid protease extensively degraded the soyprotein isolate in both processes.

For the Biogels, permeation histograms of weight fraction *vs.* elution volume, expressed as V_e/V_o, for both hydrolyzates are shown in Fig. 2. For each histogram,

weight fraction was calculated from the α-amino nitrogen following alkaline hydrolysis of the fractions. Also shown is n, which was obtained as the ratio of summed α-amino nitrogen following alkaline hydrolysis to that of α-amino nitrogen of the unhydrolyzed fractions. The values of n in Fig. 2 are similar, but slightly higher than those determined for the unfractionated hydrolyzates. For both hydrolyzates, the value of n increases as the peptides enter the gel phase to a greater extent. Regardless of this fact, there appears to be little difference between the permeation patterns of the batch and continuous hydrolyzate through the same Biogel, suggesting similar size distributions. Examination of n or $\overline{MW}_n$ as a function of V_e/V_o for all three Biogels indicated no evident relationship between size and elution position (the data are not shown). A probable explanation is that the peptides are very small; thus non-specific adsorption effects resulting from amino acid compositional differences (particularly with respect to aromatic side chain residues) predominate over the normal permeation behavior which is size-dependent.

Figure 3 is plots of mole fraction *vs.* molecular weight for the batch and continuous peptides. Mole fraction was calculated from the α-amino nitrogen distribution of the unhydrolyzed peptide fractions following permeation through Biogel P-4. The most striking features of both dispersions are their similarities and the skewness towards low molecular weight values. Roughly 90 % of the peptides in both hydrolyzates apparently have a MW <1000. The dashed curves are hypothetical exponential dispersions of mole fraction about the average molecular weight, and were calculated by the expression: mole fraction at MW = $\exp^{-}(MW/\overline{MW}_n)$. The

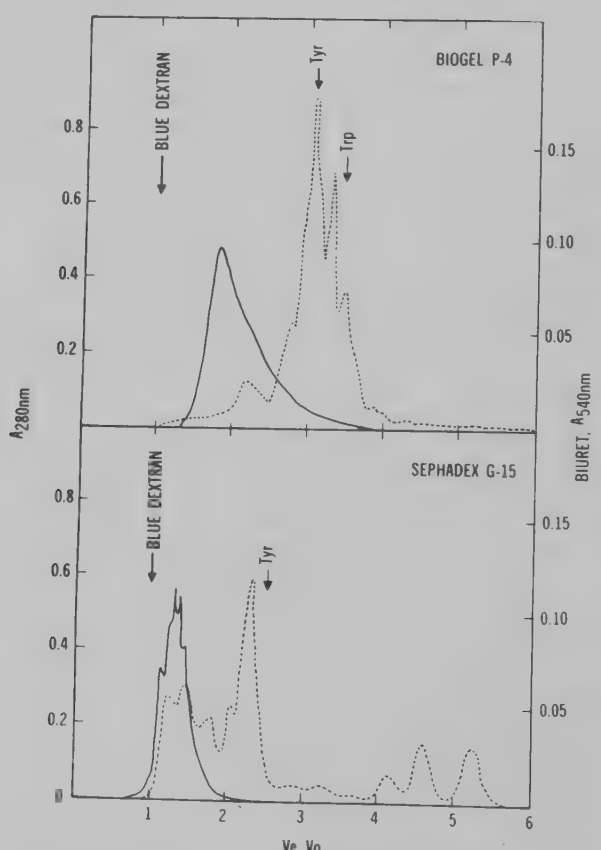


Fig. 1.–Gel permeation patterns of hydrolyzate from continuous process through Biogel P-4 (4000 MW exclusion) and Sephadex G-15 (1500 MW exclusion). Elution positions of the markers, blue dextran, tyrosine (tyr) and tryptophan (trp) denoted. See Experimental section for details.

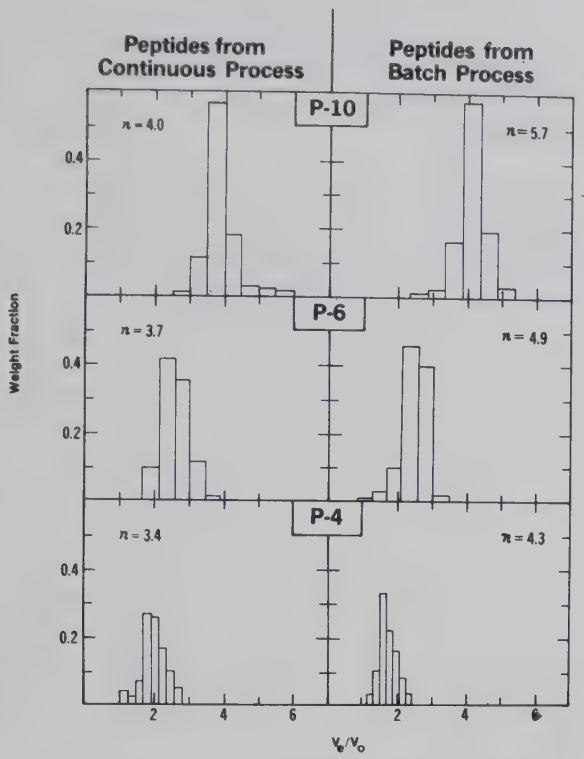


Fig. 2.–Permeation histograms of hydrolyzates from batch and continuous processes obtained for Biogels P-10 (20,000 MW exclusion), P-6 (6000 MW exclusion) and P-4 (4000 MW exclusion). Values of average chain length, n, calculated from summed fractions are denoted for each permeation.

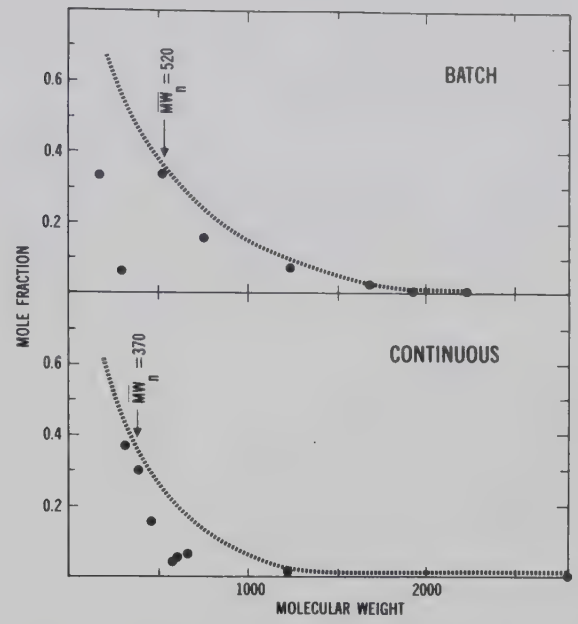


Fig. 3.–Dispersion of mole fraction vs. molecular weight for peptides from both processes obtained from Biogel P-4 permeation is denoted by dots. $\overline{MW}_n$ of both samples given as well as hypothetical dispersion (dashed line) based on exponential relationship proposed in Results and Discussion section.

dispersion, particularly for the peptides prepared by the continuous process, may obey this relationship, although more data are obviously needed for confirmation. A similar exponentially appearing dispersion has been found for the variation of peptide chain length with nitrogen distribution in the case of dialyzed soy sauce (Kirimura *et al.*, 1969), which is another enzymatic hydrolyzate of soyprotein.

We have found both the size and size distribution of peptides prepared by a batch and continuous process, witch uses a membrane reactor, similar. Organoleptically, the quality of both hydrolyzates was also similar. In the continuous process, the opportunity exists to improve organoleptic quality by regulating the peptide size and/or size distribution. Such regulation may be achieved by increasing both the size of the enzyme by means of soluble immobilization and the porosity of the membrane.

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Lesser known oilseeds: III. Nutritive value

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ABSTRACT

The nutritive values of three lesser known oilseeds *Irvingia gabonensis*, *Citrullis vulgaris* and *Parkia filicoides* were determined by feeding trials with rats. The three oilseeds are all equally highly digestible with a range of 87-99 %. The Protein Efficiency Ratio (PER) varies considerably. *Irvingia* has a low PER ranging from 0.17 – 0.49. *Citrullis* has the highest PER, range 1.23 – 1.61 while that of *Parkia* ranges from 0.88 – 1.20. The biological value (BV) and Net Protein Utilisation (NPU) follow the same pattern as the PER. *Citrullis* has the highest BV and NPU with a range of 71 – 83 and 69 – 81 respectively. The corresponding figures for *Irvingia* and *Parkia* are 73 – 75, 65 – 74 and 59 – 65, 60 – 65 respectively. On the whole *Citrullis* seems to be the most nutritious and most efficient.

INTRODUCTION

Although animal is the best source of protein, it is expensive and not within the reach of the common man. Seed is therefore his main source of protein, especially in developing countries where seeds are used mainly as food rather than animal feeds. Here Cereal is still the main source of protein and calories in developing countries but the protein content is low and of inferior quality. Oilseeds in general contain higher and better quality protein. As the population increases in these countries, seeds, especially oilseeds which are the main protein-rich crops, will continue to play more important part in the supply of protein.

These oilseeds are available in the market and are very cheap but they are not yet fully utilised. In some cases they are used as fertilisers or even wasted and so the problem of malnutrition seems to become more noticeable as the poor people depend on their regular monotonous diet of starchy roots and tubers or else highly polished cereal foods. For example in Nigeria Omololu (1972) found that almost 14 % of all deaths in the age group 0 - 4 years are directly due to malnutrition. These do not include deaths where malnutrition was a contributory factor such as gastroenteritis which caused 20 % of all deaths at these ages.

A lot is known about the nutritional qualities of certain oilseeds like soyabean, groundnut, cottonseed, etc. but very little is known about others like *Irvingia*

gabonensis, Citrullis vulgaris and Parkia filicoides. It was therefore decided to do a systematic work on these three oilseeds with a view to ascertaining their nutritive value and their possible inclusion in baby food and also in the normal diet.

MATERIALS AND METHODS

The oilseeds, *Irvingia, Citrullis* and *Parkia* were bought from a local marked and brought to the laboratory for the investigation.

(a) *Irvingia gabonensis*: The seeds were dried in the oven at 96° C for 24 hours. They were then ground up in the small sample mill. The product was collected and extracted continuously with chloroform and then air-dried.

(b) *Parkia filicoides:* These seeds were boiled for 5 hours with water. The water was decanted and cold water added. The pot was covered up and the material left to ferment for 3 days. This was then washed with water and the softened resta removed from the seeds. The seeds were dried in the oven at 90° C for 24 hours. The dried seeds were then ground in the mill and subsequently extracted with chloroform. The residue was then air-dried.

(c) *Citrullis vulgaris*: These seeds have very tough testa. They were cracked to release the seeds which were then dried in the oven at 90° C for 24 hours. They were ground in a small sample mill and the product was extracted continuously with chloroform and then air-dried.

Composition of the Test and non-protein diets

The basal protein-free diet used was that of Rippon (1959) slightly modified. It was made of the following on air-dried basis:

Sucrose	140 gm.
Butter (Danish)	100 gm.
+ Vitamin mixture	100 gm.
Non-nutritive Cellulose	100 gm.
+ Sal mixture	80 gm.
Cod-liver oil	20 gm.
Corn starch	1360 gm.

The corn-starch was added after all other ingredients had been added and thoroughly mixed together.

Salt mixture

The composition of the salt mixture was that of Rippon (1959) and was made up of the following:

NaCl	50.0 gm.
$Ca_3(PO_4)_2$	400.0 gm.
$FeC_6H_5O_7.3H_2O$	35.0 gm.
$MgSO_4.7H_2O$	80.0 gm.
NaH_2PO_4 (anhydrous)	105.0 gm.
KCl	250.0 gm.
Kl	1.0 gm.
$MnSO_4.4H_2O$	0.2 mg.
NaF	0.04 mg.

Vitamin Mixture

The vitamin mixture used in Glaxo Laboratories reported by Cuthhbertson (1957) was used. This consisted of the following vitamins per kilogramme weight of the semi-synthetic ration:

Vitamin A	4000 I.U.
Vitamin D (Calciferol)	2000 I.U.
Vitamin E (-tocopherolacetate)	280 gm.
Vitamin K	2 mg.
Thiamine	30 mg.
+ Riboflavine	30 mg.
Pyridoxine	8 mg.

Ca D-pantothenate	100 mg.
Nicotinic acid	100 mg.
Vitamin B_{12}	50 mg.
Chlorine	1000 mg.
+Pteroylglytamic acid	1.0 mg.
Biotin	0.2 mg.
Inositol	220 mg.
p-aminobenzoic acid	75 mg.

((+) Riboflavine and pteroylglutamic acid were added last.)

All the test diets were prepared by incorporating each material separately at the expense of the corn-starch in the basal protein-free diet to give 10 % protein (N x 6.25).

Both the basal protein-free diet and the test diets were each made into a thick paste with a small quantity of water to reduce spillage by the animals.

Animal Feeding and Criteria for Assessment of the Diets.

The net protein utilization (NPU) was determined by the carcass nitrogen technique of Miller and Bender (1955). The true digestibility (T. D.) of the dietary nitrogen was obtained in the same experiment by the "balance-sheet", method of Nitchell (1923-24). The biological value was calculated by dividing NPU by T. D. (Bender and Haizeldon, 1957; Campbell, 1963). The protein efficiency ratio (PER) was determined by the original method of Osborne, Mendel and Ferry (1919).

Weanling littermate male rats of a single strain (Wistar), from our own colony were used. The rats were collected at 23 - 24 days of age, numbered and housed individually in a battery of wire-screen-bottomed cages. They were weaned on to the stock diet in the experimental cages for a week so that at the commencement of the feeding trial the rats were always 30 - 31 days old and weighed between 50 - 60 gm.

The food was placed in pots specially designed to minimize spillage and scattering. Finer wire screens were placed in the plastic trays below the cages to collect the faeces and spilt food. The finer wire screens on top of each tray allowed only urine to drip through onto the tray and was finally collected in the beaker. The spilt food and the faeces were separated by hand which also allowed us to separate and discard all particles of hair.

One group of seven male rats was fed on the protein-free diet, another group was fed on the apon diet (*Irvingia gaponensis*), and the three remaining groups were placed on the locust bean (*Parkia filicoides*), water melon (*Citrullis vulgaris*) diets respectively for ten days. Water and food were given ad libitum. The weights of the animals were recorded every other day. The first 3 days were regarded as acclimatization period during which no records were kept of food consumption, and no collection of faeces was made. Collection of faeces was made daily for the last 7 days of the feeding experiment. The faeces of individual rats were pooled, dried at 105° C for 24 hours and ground into powder for faecal nitrogen estimation. Daily records of foods consumption were kept by weighing the food given and what remains together with that spilled after 24 hours.

At the end of the experimental period, animals were killed with chloroform. Incisions were made into the skull and the chest. They were dried to a constant weight in an oven with a forced draught at 105° C. The dried carcass of each rat, each including the contents of the alimentary tract as well as the small pieces of nitrogen-free filter paper used to absorb spilled blood during the dissection, was first pounded in a mortar and then transferred to a 1 - litre conical flask, 100 ml. concentrated sulphuric acid were poured over it, and after 10 minutes; 100 ml. of distilled water were cautiously added. The disintegration of the carcass began immediately on addition of the water and the digestion was well advanced in one hour without the application of external heat. After this had cooled down to room temperature, the sample in the flask was autoclaved for 3 hours at 16 lbs/sq. inch pressure as described by Rippon (1959). The hydrolysates was cooled, stirred and made up to 250 ml. 25 ml portions of the slurry were taken in triplicate for carcass nitrogen determination by the Kjeldahl-Markham (1942) procedure. The feeding trial was then repeated.

RESULTS

Table 1 gives the results of the feeding trials. The protein intake is least with *Irvingia* within the experimental period: This amounted to only about 4 - 8 gm. and

TABLE 1

Diet	Rat number	Total protein intake	Weight gain	Protein efficiency ratio (PER)	True digesti; (TD)	Biological value (BV)	Net protein utilization (NPU)
Irvingia	1	5.722	1.0	0.17	97.19	75.20	73.09
	2	8.395	2.2	0.26	94.52	73.72	69.30
	3	7.394	1.9	0.26	98.01	73.15	74.64
	4	8.238	4.0	0.49	87.74	74.42	65.30
	5	7.564	1.5	0.20	89.40	74.74	66.82
	6	5.676	2.5	0.44	94.19	79.57	74.95
	Average			0.30	93.50	75.13	70.68
Citrullis	1	14.520	25.3	1.74	91.43	83.93	76.74
	2	15.054	25.3	1.68	97.19	83.42	81.07
	3	25.020	40.5	1.62	94.94	75.01	71.21
	4	28.530	35.0	1.23	98.54	78.89	77.74
	5	22.283	30.0	1.35	94.90	80.00	75.92
	6	19.665	26.5	1.35	98.81	73.62	72.74
	7	15.738	25.3	1.61	97.21	71.79	69.69
	Average			1.61	96.15	78.09	75.02
Parkia	1	23.503	28.10	1.20	99.42	60.79	60.44
	2	20.551	19.90	0.97	98.11	59.97	61.13
	3	31.259	32.50	1.04	93.15	64.98	60.53
	4	16.496	12.5	0.76	99.00	63.18	62.55
	5	22.794	20.0	0.88	95.00	64.91	62.05
	6	17.598	15.7	0.89	98.94	65.94	65.24
	7	29.291	31.6	1.08	99.09	60.74	60.19
	Average			0.97	97.62	62.93	61.73

this therefore leads to a small weight gain (1.0 - 4 gm.). The PER was very low on the whole ranging from 0.17 - 0.49. On the other hand, *Irvingia* is highly digestible (TD 87 - 98 %) and has a high BV (73 - 75) and NPU (65 - 74).

Citrullis is similar to Parkia in many respects. Unlike *Irvingia* a high amount of protein was consumed in both cases (14 - 28 gm. and 16 - 31 gm. respectively) resulting in a high weight gain (25 - 40 gm. and 19 - 32 gm. respectively), and hence a high PER relative to *Irvingia* (1.2 - 1.7 and 0.7 - 1.2 respectively).

All the oilseeds are equally highly digestible (range of 91 - 99). *Citrullis* has a higher BV than *Parkia* (75 - 83 and 59 - 65 respectively) and also a higher NPU (69 - 81 and 60 - 65 respectively).

DISCUSSION

Irvingia, Citrulli and *Parkia* are three lesser known oilseeds which are used more or less as condiment in soup in Nigeria. *Irvingia* is usually mixed with oil, pepper and other condiment and cooked to give a slimmy consistency. This is then used as a vegetable soup. *Citrullis* is usually mixed with green vegetables such as *Talinum triangulare* and cooked with other condiments. *Parkia* is used in the least quantity in much the same way. Apart from this they are not used extensively on their own as protein-rich items of food. It is for this reason that feeding experiments

to test their nutritive values are needed; especially as there are no reports available in the literature on them.

Due to the low intake of protein from *Irvingia* the PER obtained were very low (0.17 - 0.44). *Citrullis* gave the highest PER (1.2 - 1.7), followed by *Parkia* (0.7 - 1.02). The PER obtained is similar to that of groundnut obtained by McOster (1962) usign a level of 15 % (1.81 for raw and 1.50 for roasted groundnut) and those of Buss and Goddard (1948) who used 10 % protein level and obtained PER 1.84 for boiled and 1.65 for roasted groundnut and 1.84 by Jones and Divine (1944) using 15 % level. However, they are lower than those of Cravioto and Cervantes (1965) who obtained PER value of 2.5 for rat fed raw pumpkin seed and Bressani and Arroyave who found that the PER at 10 % level using pumpkin is higher (2.28) that a 15 % level (1.80). Eklund and Agren (1970) also obtained a range of 1.8 - 2.3 for nug seeds.

The differences in the PER values for the different oilseeds is understandable. This will be due to inbalance in amino acids, e. g. the PER value of 2.5 for raw pumpkin seed for rat feed increases to 2.75 with lysine supplementation and to 2.91, closely approximating that of skim milk, with addition of soya bean meal (Cravioto and Cervantes 1965). Supplementation of a diet containing Cucurbita farinosa meal with either threonine or methionine obtained no improvement in the growth of rats (Asenjo and Goyco 1951; Bressani and Arroyave 1963). However, addition of both amino acids increased growth and improved feed and protein efficiencies (Bressani and Arroyave 1963).

In general all the oilseeds are highly digestible, the true digestibility being about 95 % on the average. They also have high BV and NPU. *Citrullis* has the highest BV and NPU (75 - 83 and 69 - 81 respectively) followed by *Irvingia* (69 - 75 and 65 - 74 respectively). The corresponding figures for *Parkia* are 59 - 65 and 60 - 65 respectively. These are all high relative to some other known oilseeds, e.g. NPU for groundnut ranges from 45 - 50 (McOster 1962 and Dreyer et al 1964) found that at 10 % protein Bambara groundnut gave a biological value of 56.0 % and digestibility of 82.6 %. For full fat soya bean meal Rippon (1959) obtained a BV 74, TD88 and NPU 66 and for the petroleum extracted meal the corresponding figures were 59, 97 and 54 respectively, and for full fat groundnut he obtained BV 73, TD 96 NPU 70 and for extracted meal 61, 95 and 58 respectively. Similarly Henry and Toothill (1962) reported BV and TD of 76.6 and 91.9 respectively for soya bean; and 76 and 83 respectively for linseed cube.

In general the higher the amount of protein consumed the lower the BV which is similar to the observation of Henry and Kon (1957) for a number of various foods. This makes direct comparison difficult but it gives a good estimate all the same.

Since the nutritional properties could be reduced by limiting amino acids experiments are on hand to study the effect of supplementation with certain amino acids and also with casein, soya bean and other well known oilseeds.

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Leaf proteins: extractability from some nigerian leaves

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ABSTRACT

Maize leaves gave a high extractability on a small scale but was poor on a larger scale. Legumes gave high values, as much as 400 kg protein/ha could be extracted up to 8 weeks and this decreased subsequently. As much as 90 % of the proteins of green vegetable leaves are extractable in some cases. As much as 300 kg protein/ha can be obtained in 5-6 weeks.

INTRODUCTION

It has now been established that protein can be extracted from green leaves using various machines and that the product has a good amino acid pattern, similar to egg and better than the best oilseeds (Pirie 1953, 1959; Oke 1971; Kohler *et al* 1968). Recently Bray (1974) showed that the process is economical. What is now important is the agronomic aspect of the work.

Forage crops produce the highest yield of protein per acre in form of green leaves producing about 3 to 10 times more crude protein per acre than the major seed crops e. g. Lucerne 2.400 Sorghum - Sudan 2070 while cereal grams like corn seed produce 780, rice 175, soybean 700 lb of protein per acre (Stahman 1968). The amino acid pattern varies only slightly with age and species so that one could utilise crops that are normally not eaten by man or those that are a nuisance such as water hyacinth. It is therefore desirable to test the extractability of protein from some local plants in order to ascertain which could best be adapted for the extraction process as well as the processing variables that may affect the protein yield.

Peasant farming is still practised to a large extent in Nigeria. The farmer grows enough food for himself and some to spare for money. When the land is exhausted he shifts to another area and allows the first one to revert to bush. By this shifting system of agriculture the farmer can do away with fertilizer, though his yields are small compared to Western standards.

Because of this factor, the first phase of our work on leaf protein was concentrated on the agronomical aspects without the use of fertilizers. This will give us an idea of the type of yield that would be expected locally.

The posho mill

Although all the results reported here were obtained using the IBP pulper and press, work has started on the adaptation of Posho Mill. The operation of this mill is similar to the IBP machine already described in detail by Pirie (1961). The mill is available in every village in Nigeria and is used mainly for grinding corn and beans. Since there is no electricity in all the villages, the mills are designed to run on diesel engines.

The material is fed into the mill through a tray on top of the feed tube. Water can be added from a drum attached to the tube if necessary. The cutter consists of blades which can be adjusted by a small wheel for fine or coarse grinding. The machine is cooled by water from a drum. Results similar to those of IBP machine have been obtained with the Posho Mill.

MATERIALS AND METHODS

The seeds of the different plants used for this experiment was obtained from the University of Ife Farm. They were planted on plots of about 15 x 15 meters. In general they were sown thicker than normal especially with the vegetables.

The crops were harvested in the morning and brought to the laboratory, which was about half a mile from the farm. About 1-2 kg samples are pulped with the IBP machine and this is spread over an area of about 450 cm^2 and subjected to pressure using 5 x 1000 kg weights. The juice was collected and analysed for nitrogen by Kjeedhal digestion. For protein-N, 10 % trichloroacetic acid was added to the juice and the precipitate is certrifuged off and analysed for N.

RESULTS AND DISCUSSION

The plants used for the large scale extraction could be divided into three categories: cereals, legumes and green vegetables.

(a) Maize was used as a typical cereal grown in Nigeria. The percentage N in the dry matter increased from 3.0 to 3.5 in the first five weeks, and then started to decrease gradually until the eleventh week, when the lowest figure of 2.5 was obtained. The true protein followed the same pattern. The percentage dry matter increased progressively from 18.1 to 24.0 in elevent weeks. The amount of protein extracted was low, ranging from 35 to 50 %.

(b) In legumes the percentage N in the dry matter and the protein-N increased up to 8 weeks before it started to decline: The total N increased from about 12.0 to 64.8 kg/ha. Over 70 % of this was extractable during the first 8 weeks. Extractability then decreased to about 40 % after 10 weeks. About 80 % of the total N extracted during the first 7 weeks was protein-N and this decreased to about 25 % after 11 weeks.

(c) The percentage N in the dry matter and the protein-N increased for up to 7 weeks and then dropped more steeply than with legumes (Fig. 1 & 2). The total N started to decrease after 9 weeks. The percentage N extracted was very high in vegetable as high as about 90 % in most, up to 8 weeks. Most of the total N extracted was true protein (about 80-90 %) up to 6 weeks, and this decreased to low values (40-50 %) in II weeks.

In the United States Khzoler (1970) and his colleagues have found that alfalfa in the best plant for protein extraction. It has the added advantage that the residue still contains enough protein to be sold as cattle feed. In India Singh (1967) and Joshi (1970) found leguminous plants like *Medicago sativa* and Vigna *sinensis* to be suitable.

In this agronomic trial the plants were divided into three categories: cereals, legumes and green vegetables.

The extractability of protein from maize leaves (cereal) on a large scale is relatively low, and yet on a small scale cereal leaves give the best results. Only the

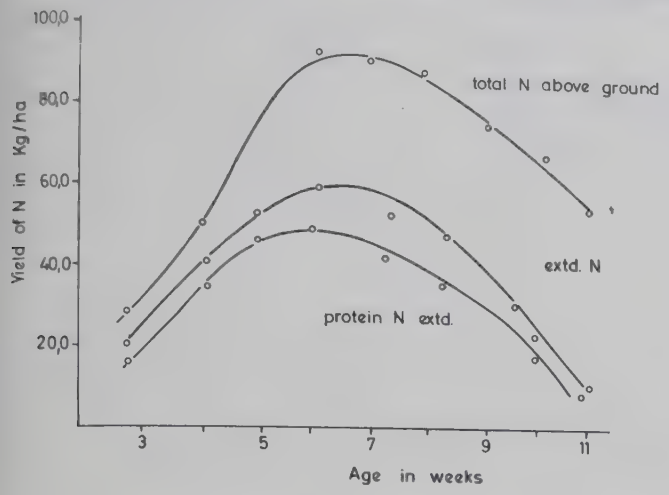


Fig. 1.–Solanum in Nigeria (unfertilised).

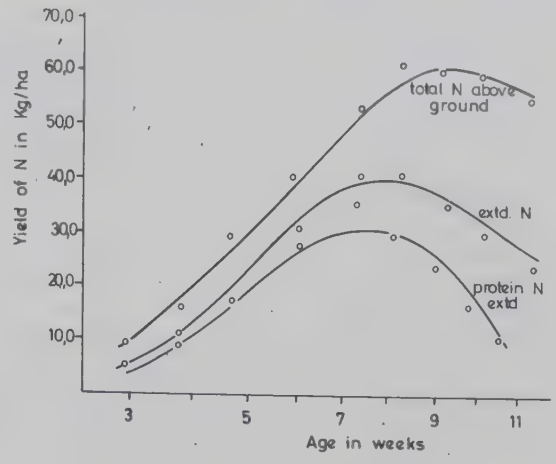


Fig. 2.–Cowpeas in Nigeria (unfertilised).

ESI type of maize has been so far used, and no fertilizers were applied. Cereals are known to give very poor yields when they are not fertilized, and this may have had some effect on the extractability. Harvesting could be done earlier if necessary. In Nigeria the stalks are left to dry out and are then buried in the soild or burned. Cereals will therefore offer a good cheap source of leaf protein. Joshi (1970) obtained and yield of 70 kg in 74 days using *Eleusine coracana.*

Legumes have the added advantage of being able to fix atmospheric N 'and so enrich the soil as well. As much as 400 kg protein/ha could be obtained in 8 weeks, or aboud 3,000 kg Protein/ha/annum. This is probably aboud thrice the amount of proteinthat would be obtained if the plants were grown for seeds only.

Only one harvest was made. It is possible to allow the plants to regrow and harvest again, Joshi (1970) in India obtained yields of up to 187 kg of extracted protein in 85 days using *Lablad niger* when cut three times (38 + 27 + 20 days intervals) whereas they obtained 220 kg when cut twice (45 + 308 IN 75 days, using a fertilizer rate of 60 kg P/ha. Using Lucerne Singh (1967) also in India, obtained a yield of 1.5 tons/ha/year without special attention to manurial treatment. Using NPK fertiliser 120 + 500 + 125 kg/ha Joshi (1970) obtained a yield of 3114 kg ptotein/ha in one year, the first harvest being done after 50 days and 5 others at 26 days intervals. They found the yield vary with the season e. g. in the rainy season they obtained a yield of 500 kg more. In the present trial attention was not focussed on the regrowth since we are only interested was not focussed on the regrowth since we are only interested at present in a suitable crop.

With *Vigna sinensis joshi (1970)* using NPK fertilizers obtained a yield of 643 kg protein/ha and with more rain could go as high as 800 kg.

The extractability of protein from legumes is high. With groundnut nearly 80 % of the protein was extracted at the peak period of 7 weeks. This decreased slowly at first and rapidly after 10 weeks to about 36 % in 11 weeks. *Vigna Unguiculata* gave a similar result by with a peak between 6 & 7 weeks. Joshi (1970) gave extractability of lucerne was higher in winter than in summer hot days. He found that if lucerne was established continuously. for 1-1 1/2 years with additional dressings of N & P fertilizers it was possible to get a yield of 3000-4000 kg/ha/year.

The decrease in extractability coincided with the period of seeding, and so far

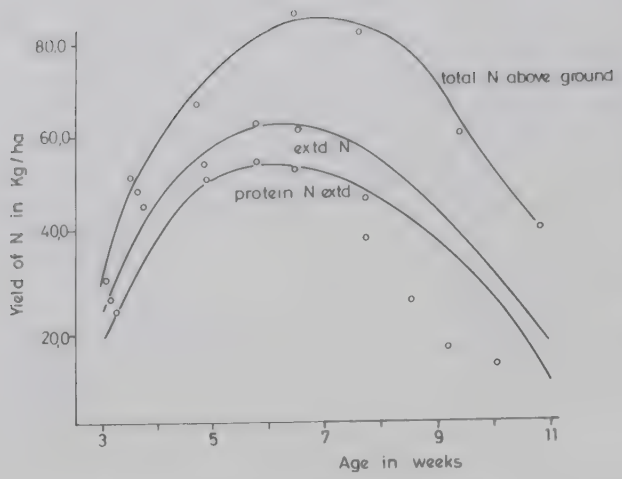


Fig. 3.–Amaranth in Nigeria (unfertilised).

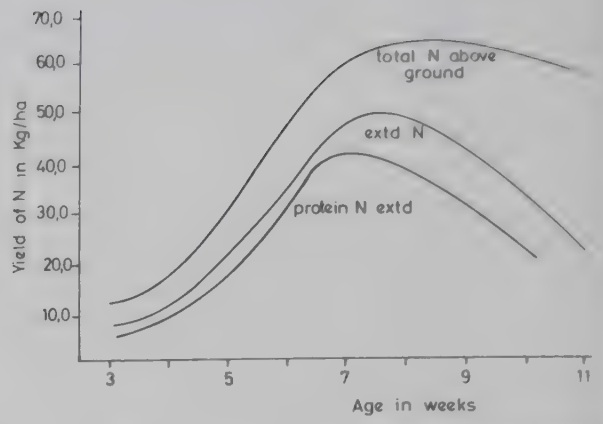


Fig. 4.–Ground nut in Nigeria (unfertilised).

maximum extraction the legumes could be planted close together, instead of adopting the usual spacing to allow for maximum seed yields, and the harvesting could be done just before seeding.

The leaves of green vegetables are very succulent and offer a good source of easily extractable protein. As much as 90 % is extractable in some cases. When the vegetables are used for soup about 30-60 % is wasted and thrown away, whereas the whole material is used in protein extraction.

Using *Spinacia oleracea* Joshi (1970) obtained a yield of 109 kkg protein, 78 kg *Coriandrum sativum* and 583 kg protein from *Amaranthus paniculatus* using N fertiliser (125 kg/ha) and hasvested twice in 90 days. In general the yield of the green vegetables used varied from 78-173 kg of protein/ha.

The green vegetables gave the highest extractability values. As much as about 90 % of the total N in *Amaranthus* can be extracted by the fourth week and this decreases gently at first and then rapidly after 10 weeks to 53 % in the tenth week and 41 % in the eleventh week. Similar results were obtained for *Solanum incanum, S. nodiflorum, S. macrocarpon & Celosia argentea.* As much as 370 kg/ha protein could be obtained from *Amaranthus* in 5-6 weeks. Joshi (1970) seemed to obtain a poor extractability for the vegetables he used e. g. 53 % for Spinacia and 43 % for *Coriandrum.* The only vegetable in this trial that gave a poor extractability was *Veronina amygdalina.* This contains a lot of saponin it foams profusely on extraction. The presence of phenols is also suspected due to the browning after extraction. It is known that the presence of phenols and their oxidation products affect the extractability of protein from leaves) cohen *et al* 1956, Pirie 1961, Loomis & Batailee 1966) and this may account for the low extractability (58 %) of *Vernonia* relative to the other vegetables.

In general the extractability of protein starts decreasing after 4 weeks with the vegetables, after 7 weeks with legumes and after 5 weeks with cereals, in spite of the fact that the protein content of the leaves is still increasing. This has been reported also by Crook & Holden (1948), Byers (1961) Pleshkov and Fowden (1959) and Joshi (1970). Since the dry matter is also increasing at the time the extractability is decreasing, it is possible that this leads to increased difficulty in the disruption of the cells of the leaves and hence decreases extractability. The nitrogen content usually starts decreasing after 10 weeks due to sudden cessation of protein synthesis whilst proteolysis continues (Arkcoll 1970). This is followed by wilting and yellowing of the leaves caused by intense proteolytic activity of leaf protease (McPherson 1952, Singh 1962).

TABLE 1

Extractability of protein from some Nigerian Leaves

	Age wks.	% Dry matter (DN)	% N DN	Total N Kg/ha (TN)	TN Extracted Kg/ha	Protein N (PN) Extracted Kg/ha	% TN Extracted	% PN Extracted
					Vegetables			
	3	7.1	3.4	31.3	23.2	20.1	74.0	63.9
	4	7.5	3.6	52.6	47.0	40.0	88.9	76.0
	5	9.8	4.0	63.4	53.1	48.5	83.7	76.5
	6	9.7	4.4	77.8	61.5	53.2	79.0	57.8
Amaranthus	7	11.7	4.9	83.6	58.5	50.1	70.0	59.9
	8	12.1	4.9	77.7	52.0	41.6	66.9	53.5
	9	15.5	4.6	68.3	40.2	36.3	58.9	53.1
	10	18.2	4.3	56.5	30.0	24.2	53.1	42.8
	11	18.5	3.9	38.6	16.0	10.3	41.5	26.7
	3	7.9	4.6	29.5	17.1	15.3	58.1	52.8
	4	8.8	4.7	48.4	32.8	29.0	67.8	59.9
	5	9.1	4.8	67.3	54.8	50.1	81.5	74.4
	6	9.6	5.0	75.4	53.2	49.9	70.5	66.2
Celosia	7	9.8	4.8	81.9	50.9	48.6	62.1	59.4
	8	10.6	4.7	74.6	42.8	39.1	57.4	52.4
	9	13.2	4.7	63.2	32.9	25.0	51.8	44.3
	10	15.1	4.4	54.2	26.4	22.7	48.5	41.7
	11	15.5	4.3	41.3	16.5	13.3	39.9	33.2
	3	9.6	4.5	31.5	22.1	18.5	70.3	58.6
	4	10.7	5.0	50.1	40.7	35.8	81.3	71.5
	5	12.5	4.5	69.0	50.5	44.4	73.2	64.3
	6	14.0	4.3	83.4	51.0	43.6	61.2	52.3
Solanum macrocapon	7	16.5	4.3	90.3	49.8	40.0	55.1	44.3
	8	18.1	4.1	82.3	39.8	32.2	48.3	39.1
	9	20.3	3.5	75.3	31.3	23.6	41.6	31.3
	10	22.3	3.3	61.2	22.2	15.1	36.3	24.6
	11	24.4	3.3	53.5	14.0	10.9	26.2	20.3
	3	7.0	4.8	30.1	20.9	17.6	69.5	58.6
	4	7.8	5.1	52.0	41.7	37.6	80.1	72.5
	5	9.1	5.5	66.3	47.4	44.0	71.5	66.3
	6	10.5	5.7	85.4	53.4	44.6	62.5	52.2
Solanum incanum	7	11.4	5.5	87.5	47.4	42.2	54.2	48.2
	8	12.8	5.3	75.2	33.3	20.4	44.3	39.1
	9	13.5	5.0	72.1	29.0	22.8	41.5	31.6
	10	16.1	4.6	63.1	22.2	17.2	35.2	27.2
	11	17.3	4.3	50.4	14.7	11.3	29.3	22.4

The fall in yield of extractable N makes the time of harvesting critical e.g. in *S. macrocarpon* the yield at 5 weeks was 69.0 KG N/ha and at 6 weeks increased to 83.4 kg, and 90.3 kg in 7 weeks. Harvesting at the wrong time, say at 5 weeks or after 8 weeks may therefore lead to a significant loss of protein. This loss becomes more significant because at this stage extractability also decreases rapidly.

Apart from N fertilisers which have been found to boost up the yields of

extractable protein several times over, other factors such as species, age at harvest, climate, seed rate are also important. Arkcoll (1970) has reported animal yields of up to 1200 kg of protein/ha with legumes and 200 with other crops (raddish, mustard etc) & suggested that up to 3000 - 5000 kg were possible/in the wet tropics.

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Nutritive value of leaf protein: a note on the comparison of *in vitro* and *in vivo* methods

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ABSTRACT

The nutritive value of leaf protein from wheat was determined by rat assay and by enzymatic hydrolysis with pepsin/ trypsin and pepsin/pancreatin methods. The two enzymatic methods agreed very closely, (89.7 and 90.3 respectively) and correlate very well with the true digestibility (89.64) using the rat assay technique. The biological value using rat varied from 61-72 with an average of 69.06 and the net protein utilisation varied from 55-65 with an average of 61.84. These coupled with the high PER (1.4–2.1) makes leaf protein a promising protein-rich supplement both for human and animal nutrition. The enzymatic hydrolysis technique is much faster and easier and could be a very useful method for assaying nutritive value of protein foods.

INTRODUCTION

Amino acid analysis has shown that leaf proteing from different sources are similar in composition (Chibnall 1939) though recently some differences are noted in the basic and sulphur-containing amino acids (Byers 1970; Oke 1971). The general result is that leaf protein should *a priori* be as nutritious as animal protein in view of the similarity in the pattern (except for a consistently marginal methionine content). It is more nutritious than the best seed protein and contains higher methionine than even Opaque-2 and Floury-2 maize. However, these assumptions have to be confirmed by animal experiments.

Earlier experiments with animals have given low biological values due to improper washing, poor extractability and presence of growth inhibitors. Thus the average digestibility for cytoplasmic and chloroplastic protein fractions were 61 % and 46 % respectively compared to 75 for soyabean (Davies *et al* 1952). The results become worse when the NPU is determined e. g. the corresponding figures for NPU were 45, 28 and 72 respectively.

Subsequent trials with rats have shown that leaf protein was inferior to dried skimmed milk powder, similar to cottonseed meal and superior to groundnut meal (Duckworth and Woodham 1961).

Animal experiments take such a long time and are laborious. When screening of new protein foods a shorter and more rapid method is desired. Since amino acid analysis only gives and idea of whether a protein might be suitable without indicating how available the amino acids are, an *in vitro* digestion using digestive enzymes might be more suitable. This will reduce the labour and the size of sample that is used for analysis. It is desirable to find how this method correlates with the *in vivo* method using animals.

MATERIALS AND METHODS

The leaf protein was subjected to enzymatic digestion using the methods of Saunders and Kohler (1971). For the Pepsin/ trypsin digestion 1 gm of the leaf protein was suspended in 1 ml. of 0.01N HCl and shaken gently for 48 hours at 35° C. After centrifuging the residue was re-suspended in 10 ml of water and 10 ml 0.1M sodium phosphate buffer, pH 8.0 and treated with 5 mg of trypsin. After shaking at 70° F for 16 hours it was again centrifuged, the residue washed, air-dried and analysed for nitrogen.

For Pepsin/pancreatin digestion about 250 mg of leaf protein was suspended in 15 ml of 0.1N NCl containing 1.5 mg pepsin and gently shaken at 35° C for 3 hours. It was neutralised with 0.5N NaOH, treated with 4 mg pancreating 0.005M sodium azide) and shaken again for 24 hours at 35° C. It was centrifuged and the residue was washed and used for nitrogen determination.

The basal protein-free diet used was a modification of that of Rippon (1959). The composition of the diet is shown in next table:

Composition of Basal Protein-Free Diet

Ingredient	Amount (g)
Sucrose	140
Butter (Danish)	200
Vitamin mixture[1]	100
Non-nutritive Cellulose	100
Salt mixture[2]	80
Cod-liver Oil	20
Corn Starch[3]	1360

1. Vitamin mixture (3) [per kg wt of semi synthetic ration]: Vitamin A, 4000 I.U.; Vitamin D (Calciferol), 2000 I.U.; Vitamin E (a-tocopherol-acetate), 280 mg; Vitamin K, 2 mg; Thiamine, 30 mg; Riboflavin*, 30 mg; Pyridoxine, 8mg; Ca D-pantothenate, 100 mg; Nicotinic acid, 100 mg; Vitamin B_{12}, 50 mg; Chlorine, 1000 mg; Pteroylglutamic acid*, 1.0 mg; Biotin, 0.2 mg; Inositol, 220 mg; p-aminobenzoic acid, 75 mg; (*Riboflavin and pteroylglutamic acid were added last). 2. Salt mixture composition (2); NaCl, 50 g; $Ca_3(PO_4)_2$, 400 g; $FeC_6H_5O_7.3H_2O$, 35 g; $MgSO_4.7H_2O$; 80 g; NaH_2PO_4 (anhydrous), 105 g; KCl, 250 g; Kl, 1 g; $MnSO_4.4H_2O$, 0.2 g; NaF, 0.04 g. 3. The corn-starch was added after all other ingredients had been added and thoroughly mixed together.

All the test diets were prepared by incorporating each material separately at the expense of the corn-starch in the basal protein-free diet to give 1-% protein (N x 6.25). Both the basal protein-free diet and the test diets were each made into a thick paste with a small quantity of water to reduce spillage by the animals. The NPU was determined by the carcass nitrogen technique of Miller and Bender (1955). The true digestibility (T. D.) of the dietary nitrogen was obtained in the same experiment by the "balance-sheet" method Mitchell (1924). The biological value was calculated by dividing NPU by T.D. (Bender and Haizeldon, 1957; Campbell, 1957). The PER was determined by the original method of Oshorne, Mendel and Ferry (1919). Weanling litter mate male rats of the Wistar strain from our own colony were used. The rats were collected at 23-24 days of age, numbered and housed individually in screen-bottomed cages. They were weaned to the stock diet in the experimental cages for a week so that at the commencement of the feeding trial the rats were always 30-31 days old and weighed between 50-60 gm.

The food was placed in specially designed pots to minimize spillage and scattering. Finer wire screens were placed in the plastic trays below the cages to collect the feces and spilled food. The finer wire screens on top of each tray allowed only urine to drip through onto the tray which was finally collected in a beaker. The spilled food and feces were separated by hand which also allowed us to separate and discard all particles of hair.

One group of seven rats was fed on the protein-free diet, another group was fed the leaf protein diet, for ten days. For comparison purposes, casein at the 10 % protein level was also fed to another group of seven male rats for ten days. Water and food were given *ad libitum*. The first three days were regarded as an acclimatization period during which no records were kept of food consumption, and no collection of feces was made.

Collection of feces was made daily for the last 7 days of the feeding experiment. The feces of individual rats were pooled, dried at 105° C for 24 hours and grouped into powder for fecal nitrogen estimation. Daily records of food consumption were kept by weighing the food given and that which remained together with the amoung spilled after 24 hours.

At the end of the experimental period the animals were sacrified and incisions made into the skull and chest. Animal carcasses were dried to a constant weight in an oven with a forced draught at 105° C. The dried carcass of each rat, including the contents of the alimentary tract as well as the small pieces of nitrongen-free filter paper used to absorb spilled blood during the dissection, were first pounded in a mortar and then transferred to a 1 liter conical flask. 100 ml of concentrated sulphuric acid were poured over it, and after 10 minutes, 100 ml of distilled water were cautiously added. The disintegration of the carcass began immediately on addition of the water and the digestion was well advanced in one hour without the application of external heat. After this had cooled to room temperature, the same in the flask was autoclaved for 3 hours at 16 P. S. I. pressure as described by Rippon (1959). The hydrolysate was cooled, stirred and made up to 250 ml. 25 ml portions of the slurry were taken in triplicate fro carcass nitrogen determination by the Markham Kjeldahl procedure (1942). The feeding trial was then repeated.

RESULTS AND DISCUSSION

The PER of leaf protein varies from 1.4-2.1 as shown in Table 1. The protein is highly digestible with the true digestibility (T. D.) varying from 86-93 %. The biological value (B. V.) varies from 61-72 and the NPU 55-65. The enzymatic digestion is more consistent. With Pepsin/Trypsin the value obtained was 89.72 while Pepsin/Pancreatin digestion gave 90.31.

Most of the preliminary work on the best conditions for extracting proteins from leaves and preserving them have been done using test animals to ascertain the difference in quality of the protein prepared under various processing conditions. The leaf protein used for this experiment was prepared by the standard method and freeze-dried. Using the PER as criterion the average value obtained was 1.79 with a range of 1.46-2.7. This compares favourably with the results of other workers (Duckworth and Woodham; 1961; Buchanan, 1969; Suba Rau and Singh, 1970). The PER could be improved considerably by supplementation with methionine (Shurpalekar *et al*, 1969; Singh, 1970). However, the important thing is that leaf protein can act as a suitable supplement for cereal foods due to its high lysine content, bearing in mind that it is not an item of food by itself but a supplement.

Leaf protein is highly digestible with an average true digestibility (TD) of 89.64, (range 86-93 %). Low digestibility due to improper processing has been atributed to the poor quality of leaf protein earlier on reported by Davies *et al* (1952). Since then considerable improvement has been achieved in the preparation of leaf protein, and Buchanan (1969) has reported a range of 53-81 %, Henry and Ford (1965) gave a range of 70-90 %. The present range is the highest so far reported and this makes leaf protein as digestible as the oilseed meals, e. g. Soyabean meal 91,

TABLE 1

Feeding Trials With Leaf Protein

Diet	Rat No.	Total protein intake	Weight	PER	TD	BV	NPU
Leaf protein	1	6.796	12.30	1.81	86.76	72.40	62.81
	2	5.243	10.20	1.95	90.19	61.99	55.91
	3	5.529	12.01	2.17	87.15	76.96	67.07
	4	7.170	13.70	1.91	90.34	62.43	56.40
	5	5.803	8.50	1.46	86.97	71.71	62.37
	6	5.082	8.50	1.62	93.40	67.29	62.85
	7	6.175	9.80	1.59	92.67	70.67	65.49
		Averages:		1.79	89.64	69.06	61.84
Casein, 10 %	1	10.5	29.4	2.8	85.9	85.7	73.5
	2	8.4	22.6	2.7	83.6	90.5	75.5
	2	9.6	27.3	2.9	84.1	84.4	72.4
	4	11.1	34.2	3.0	86.8	84.4	73.2
	5	7.9	21.2	2.7	86.4	83.1	71.9
	6	7.1	19.9	2.8	86.7	82.3	72.8
		Averages:		2.8	85.6	85.1	73.2

TABLE 2

In Vitro *Digestion of Leaf Protein*

Pepsin/Trypsin	Pepsin/Pancreatin
89.7	90.3

linseed cake 83 % Henry and Toothil (1962) and lucerne, trefoil and earthnut meals in which the TD varies from 72-96 %.

The BV obtained appears to be low on the average (69.96 rante 61-72) especially when compared to those reported recently by other workers such as 80.3 by Henry and Ford for leaf protein from wheat, with a range of 53-83 for several species Buchanan (1969) obtained a range of 80-85. However due to the low digestibility obtained his values of NPU (45-69) are lower than those obtained in this work (55-65). He found that these values could be increased by adequate washing with water and adjustment of pH before drying.

Higher values were obtained by using enzymatic digestion method for the biological value of leaf protein than animal tests. Both pepsin/trypsin and pepsin/pancreating gave very close agreements with the latter giving a consistently higher value. (78.81 and 80.13 respectively). However the results are of the same order of magnitude as those obtained by Akeson and Stahmann (1965) using the amino acids released by a pepsin followed by pancreatic digestion. Using 12 food proteins which covered the entire range of protein quality they observed and excellent correlation ($r = 0.990$) the biological values obtained from rat feeding trials. Using papain digestion Buchanan (1969) reported a range of 20-76 and found that this method correlated best with the TD, followed by pepsin/pancreatin

digestion. It appears that the enzymatic methods are not as sensitive to unavailability of some of the essential amino acid like lysine as the rat assay procedure and so they tend to overestimate.

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Alternative uses of crab for human consumption

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ABSTRACT

A laboratory method of extracting the edible portion of the crab has been developed and various products were prepared from it. The fresh or frozen crabs are sliced and are then subjected to hydraulic pressure. The extractable portion which includes the meat and other components are pressed out and collected giving a total yield of 53 % which is more than double that obtained from picking the meat by hand or by machine. This portion is homogenized and freezedried. The freezedried product is milled to produce a homogeneous fine powder. The crab powder is of high nutritional value as judged by aminoacid analysis. Various products can be prepared from it such as soups, dips and extruded food items. The dried crab powder is flavorful and renders itself suitable as a flavoring compound and as a fortifying agent. One of the many advantages of the method is that crabs which so far were not utilized due to the small size or due to the inadequate meat content can now be utilized as food for man or as a feed ingredient for animals.

INTRODUCTION

Conventional species of marine animals are becoming scarce due to overexploitation. There is, however, an increasing demand for seafood throughout the world, and to meet this demand, man must seek resources that are still underutilized or utilize efficiently the waste obtained from seafood processing.

Crabs are present in all the seas of the world. They may vary in size but they have one characteristic in common and that is that their body consists of edible and nutritious components. In many parts of the world some crabs are considered to belong to the unutilized species and are ignored. They are classified as undesirable, unfamiliar species of no commercial value, and if caught incidentally they are thrown back to the sea. Unlike the shrimp and the lobster it is difficult to separate the shell from the edible portion of the crab. This constitutes a difficulty and it is one of the reasons that crabs in general are still considered to be an underutilized species. On the other hand certain kinds of crabs are very small in size and cannot be utilized using the conventional methods of meat picking, yet their body contains rich sources of nutrients.

The waste generated by the crab industry is considerable in view of the large percentage of unutilized portions of the crab body, such as the carapace, viscera, soluble components and flesh which are lost during the processing operations. If we assume a 20 % yield of meat from the crab body it means that for every 1000 kgs of crabs caught 800 Kgs of waste is produced.

Various workers have evaluated crab meal which consists of dried shell, viscera and part or all of the flesh. Parkhurst and Lubitz (1942) analyzed crab meal for various nutrients and found it to be high in minerals. Meyers and Rutledge (1971) discussed shrimp meal which consists mostly of the heads and hulls of the shrimp, as a protein supplement for poultry and swine. Crayfish waste meal and its nutritional value was studied by Lovell et al. (1968). The biological value of crab meal has been reported to be equal to fish meal once the chitinous nitrogen has been removed (Lubitz et al. 1943) and (Lovell et al. 1968).

Meat picking operations by hand or machine may yield 20 % crab meat in crabs such as the Red crab. The remaining portion is made up of the shell and another part which consists of the various organs and other tissues remaining following the meat picking operation.

The objective of this study was to totally utilize the crab for human consumption. For that we used the Red crab, a species found off the coast of the Atlantic ocean from Nova Scotia to the Gulf of Mexico. This species (*Geryon quinquedens*) should not be confused with the pelagic Red crab (*Pleuroncodes planipes*). This present study has shown that the waste generated has a great potential as a source of nutrients, proteins, vitamins, minerals and can be utilized both as food for humans and as feed for animals. Waste treatment in this way will provide an economic recovery of potentially valuable by products. Such a utilization of the waste will solve the problem of finding ecological means of disposing crab waste, and at the same time it can be profitably converted into food and feed.

EXPERIMENTAL

Sample preparation

A simple laboratory procedure employing a hydraulic press (Carver) was used to extract the portion of the crab that could be pressed out. The crabs were first sliced into four segments, placed in a nylon net and pressed between two aluminum plates. The pressure was raised gradually from 450 lbs. per sq. inch to 5,000 lbs. The juicy portion flowed out first followed by the fleshy portion. Preliminary work done also showed that deboning machines and strainers could be used to extract the waste left on the crab following the meat picking operation. Recently Spinelli et al. (1974) also reported the use of deboning machines to prepare a pulp from small crabs as an aquacultural feed ingredient.

Proximate analysis

Samples were analyzed for moisture, protein, ash and fat using the AOAC (1970) procedures.

Aminoacid composition

The aminoacid analyzer (Technicon Corp. Model 1300) was used.

Crab powder preparation

The extract which was collected following the pressing process or after passing through a deboning machine was homogenized in a Waring blendor and spread uniformly in stainless steel trays and freezedried in a Virtis Model USM15 freezedrier. The freezedried product was then ground in a mortar, packaged and stored at -20° C.

Preparation of soups with crab flavor

Campbell's condensed soup (vegetable, tomato, onion) were used. Twenty parts of crab powder were added to 100 parts of condensed soup and mixed in a Waring blendor. One part of water was added

to each part of condensed soup. The final concentration in the soup was 10 % crab powder giving a total of about 5 % protein.

Extruded product preparation

Milled defatted corn grits and crab powder, 10 %, were extruded in a 1/4" diameter Brabender Model 3002 extruder.

Crab dip preparation

Crab powder was mixed well with sour cream, 1 : 10.

RESULTS AND DISCUSSION

Red crabs were steamed for twenty minutes and then the meat was handpicked. Figure 1, shows the waste following handpicking of the meat. The yield of the meat is 21 % with 12 % losses occurring during the preparation. This leaves a waste of 67 %, which when further treated using the pressing method yields a final shell waste chitinous residue of 47 % and an extractable portion of 20 % which can be further dried into a powder and utilized. In this case the total yield that can be obtained is about 40 %; 21 % from the meat picking operation and 20 % from further extracting the edible portion from the shell waste. If, however, the meat picking operation is omitted and the crabs directly undergo the pressing operation, the extracted portion can reach 53 % yield leaving 47 % of the original crab in the form of a final shell waste, (Figure 2).

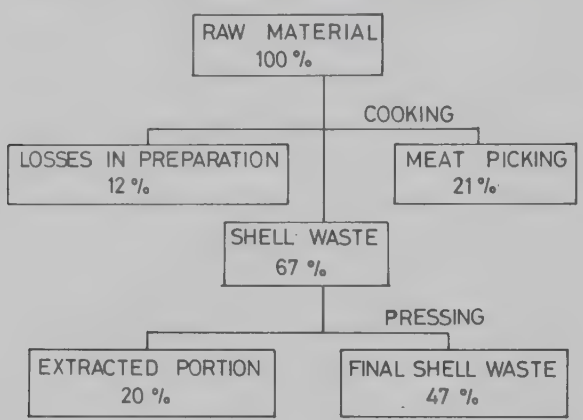


Fig. 1.–Recovery of waste following handpicking.

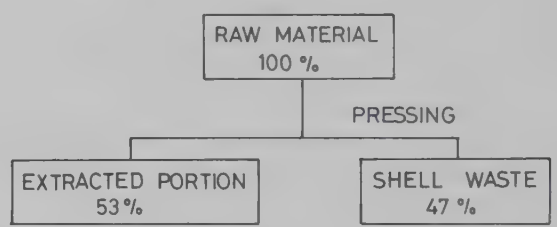


Fig. 2.–Recovery of total extractable components of the crab.

Figure 3 shows the final yields of dried shells and crab powder following drying. The crab powder obtained is 10 % of the original wet weight of the crab while the dried shells make up 16 % of the original weight of the crab.

Figure 4 shows the composition of the crab on a wet basis. The dry matter makes up 26 % of the crab, of which 16 % is the shell and 10 % is the extractable portion.

The proximate analysis of the crab powder on a wet and dry basis is shown in Figure 5. The protein content is about 50 %.

Figure 6 shows the aminoacid composition. As anticipated it is high in the essential aminoacids shown in asterisks.

The crab powder obtained is a functional product, rich in nutrients and extremely flavorful. These desirable characteristics can be utilized in the preparation of various products. Many canned soups in the market such as onion, tomato and potato soups were tested for acceptability after a certain concentration of crab powder was added. A concentration of 10 % crab powder added to the soups produced a delicious product very different form the original vegetable soup.

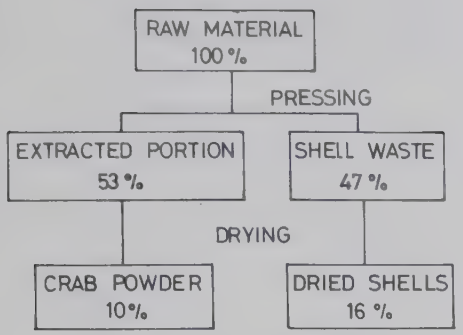


Fig. 3.–Processing into crab powder.

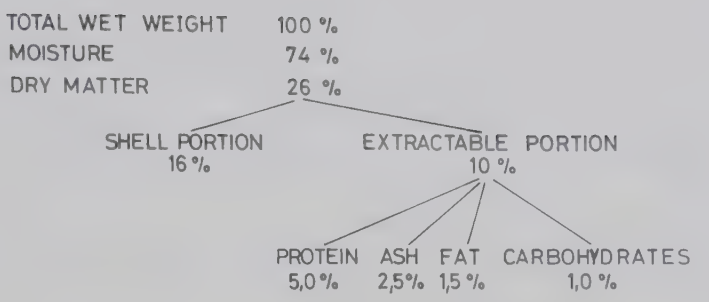


Fig. 4.–Composition of red crab

%	MOISTURE	PROTEIN	ASH	FAT	CARBOHYDRATES
WET BASIS	12,7	45,5	21,3	11,9	8,9
DRY BASIS	0,0	52,2	24,4	13,7	9,7

Fig. 5.–Proximate analysis of crab powder.

ASP	10,1	MET*	2,5
THR*	4,8	ISL*	5,7
SER	4,2	LEU*	7,5
GLU	12,4	TYR	2,6
PRO	4,4	PHE*	3,0
GLY	5,9	LYS*	9,2
ALA	6,5	HIS	2,5
VAL*	5,6	ARG	10,6
CYS	1,4	TRY*	—

Fig. 6.–Amino acid composition of the red crab.
(grams/16 grams N).

It had a distinct and acceptable crab flavor. By incorporating 10 % crab powder the protein content of the soup rose above the 5 % level, since crab powder contains about 50 % protein. Consequently, a vegetable flavored soup can be converted to a delicious "marine" flavored soup with high biological value.

Crab powder was also added to various dip preparations at a level of 20 %. The dips obtained were identical to the control samples, that is, without crab powder as far a consistency, texture and appearance was concerned. However, the samples with crab powder added had an acceptable crab flavor and were rated high. A dip with plain sour cream can be made to have a delicious and appetizing crab flavor.

Crab powder at a level of 20 % was added to corn meal with 10-15 % moisture and passed through an extruder after having optimized the extrusion parameters. Figure 7 shows the results of the taste panel tests.

	CRAB DIP	CRAB SOUP	CRAB EXTRUDATE
TASTING SCORE[2]	8,1 ± 0.7	8,0 ± 0,9	6,7 ± 0,6

1. NO. OF PANELISTS 25.
2. MEAN ± STANDARD ERROR BASED ON A NINE POINT HEDONIC SCALE.

Fig. 7.–Taste panel tests[1].

The process can utilize more of the crab body, doubling the yield of the edible portion of the crab. The method can be used as a supplementation process to hand picking or machine picking of crabs. Such a process can be extended to all kinds of crabs and in general crustacean. Waste from the shrimp, lobster, crayfish etc. industries could be utilized in the same manner. Small sized crabs which cannot be utilized otherwise can be processed in the above manner. Frozen as well as frech crabs can be used. Furthermore, crabs which have been deformed during transportation could be utilized, in this case for feed purposes.

The crab powder obtained can be used for fortification oı low protein foods

such as in potato soups, onion soups, tomato soups, dips, extruded corn or wheat products. It offers also the advantage of imparting crab flavor to foods such as those mentioned above. The crab powder can also be used in aquaculture, as a source of protein, partly replacing the other proteins present in the commercial diet. The chitinous shell residue can be used as a raw material for chitosan and chitin derivatives (Pariser and Brock, 1972).

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Production d'isolats de soja par ultrafiltration et diafiltration

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ABSTRACT

The traditional technology for production of soybean protein isolates determines a marked denaturation of proteins with a proportional loss of solubility of the final product.

In this paper we report the results of purification experiments carried out on aqueous protein extracts through a combined process of ultrafiltration and diafiltration.

The extracts were obtained from defatted flakes, desolvetized at low temperature. The experiments have been carried out on both laboratory and pilot-plant scale. The protein isolate obtained with this process presents an activity of trypsin inhibitors slightly greater than the best commercial isolate; however it presents a lower content in saponins and a higher urease activity. The low denaturation of proteins was confirmed by electrophoretic analysis. The Nitrogen Solubility Index of the isolate obtained with the new process was about 93 %, while for the best commercial preparations it rarely exceeds 68 %.

INTRODUCTION

La technologie traditionelle de production des isolats de soja consiste en une extraction par l'eau en milieu alcalin des flocons dégraissés et toastés, la séparation des matières insolubles, la coagulation des protéines à leur point isoélectrique, le rinçage à l'eau du coagulum ainsi obtenu, son éventuelle dissolution par une solution d'hydroxhyde de sodium ou de potassium à pH neutre, puis le séchage (Wolf et Cowan, 1971).

Cette technologie de base, très largement employée, et qui fait l'objèt de nombreux brevets, se prête à quelques remarques. Il est bien connu que les légumineux en général, et le soja en particulier, contiennent un certain nombre de facteurs antinutritionnels, soit de nature protéique (inhibiteurs de trypsine et hémagglutinines) que de nature non-protéique (saponines). La necessité d'inactiver ces facteurs par denaturation thermique oblige à effectuer l'extraction des protéines à partir de flocons de soja toastés; le test analytique indirect qui fournit la preuve que la dénaturation a eu lieu est représenté par le dosage de l'activité de l'uréase

(Anonymous, 1973). Cette inactivation thermique, comme le demontre d'ailleurs la littérature récente n'est que partielle (Wolf et Cowan, 1971): elle a lieu pour les hémagglutinines (qui d'ailleurs n'arrivent pas a franchir l'éstomac puisqu'elles sont inactivées par la pepsine) (Liener, 1958), mais non pour les saponines, qui sont thermostables et a lieu partiellement pour les facteurs antitrypsiques. Il semble en effect que ces derniers, pris dans leur ensable, ne sont dénaturés par la chaleur que d'une façon temporaire et donc réversible (Kunitz, 1947). De plus, l'un des deux inhibiteurs de trypsine mieux étudiés, semble être thermostable (Birk, 1961). Si le traitement thermique des flocons ne permet donc pas une inactivation efficace et complète des facteurs antinutritionnels, il provoque une dénaturation partielle des protéines (Belter et Smith, 1952) qui s'ajoute a celle due à la coagulation acide (Catsimpoolas et al., 1969). Le troisième reproche que l'on peut faire à la technologie traditionnelle consiste en la perte, dans le sérum, des protéines qui ne sont pas coagulées à pH 4,5.

Les possibilités offertes par l'ultrafiltration nous ont conduit à essayer ce procédé pour la purification des protéines de soja, comme il est déjà réalisé industriellement pour les protéines de lactosérum.

La plupart de protéines de soja sont des globulines à poids moléculaire supérieur à 50.000, tandis que les composés indésirables qui les accompagnent dans l'extrait aqueux, comme les saponines, les facteurs antitrypsique, le raffinose, le stachiose et le verbascose cause de la flatulence, etc. sont des composés à plus faible poids moléculaire.

Le but que nous nous étions fixé était de réaliser par ultrafiltration, et non par coagulation acide, la purification de l'extrait protéique aqueux obtenu à partir de flocons dégraissés et non toastés, afin d'obtenir un isolat dont la dénaturation était moindre.

MATÉRIEL ET MÉTHODES

L'extraction des protéines at été effectuée à partir de flocons dégraissés industriellement à l'héxane et dont le solvant avait été éliminé par évaporation spontanée à la température de la pièce.

Une suspension de 1 Kg de flocons par 10 Kg d'eau a été homogenisée à pH 8,5 à l'aide d'un appareil Ultraturrax pendant 30'. Le surnageant obtenu de la décartation centrifuge de la suspension a été employé dans les essais d'ultrafiltration qui ont été effectués soit à niveau de laboratorie qu'à niveau pilote.

Les essais de laboratoire ont été effectués à l'aide d'un appareil d'ultrafiltration SARTORIUS type SM 165-25, qui ne fournit pas de résultats quantitatifs extrapolables à des appareillages industriels, mais il se prête à la comparaison de différents membranes. Nous avons pris en considération cinq types de membranes, dont les caractéristiques figurent dans le tableau 1.

TABLEAU 1

Caractéristiques des membranes employées dans les essais de laboratoire

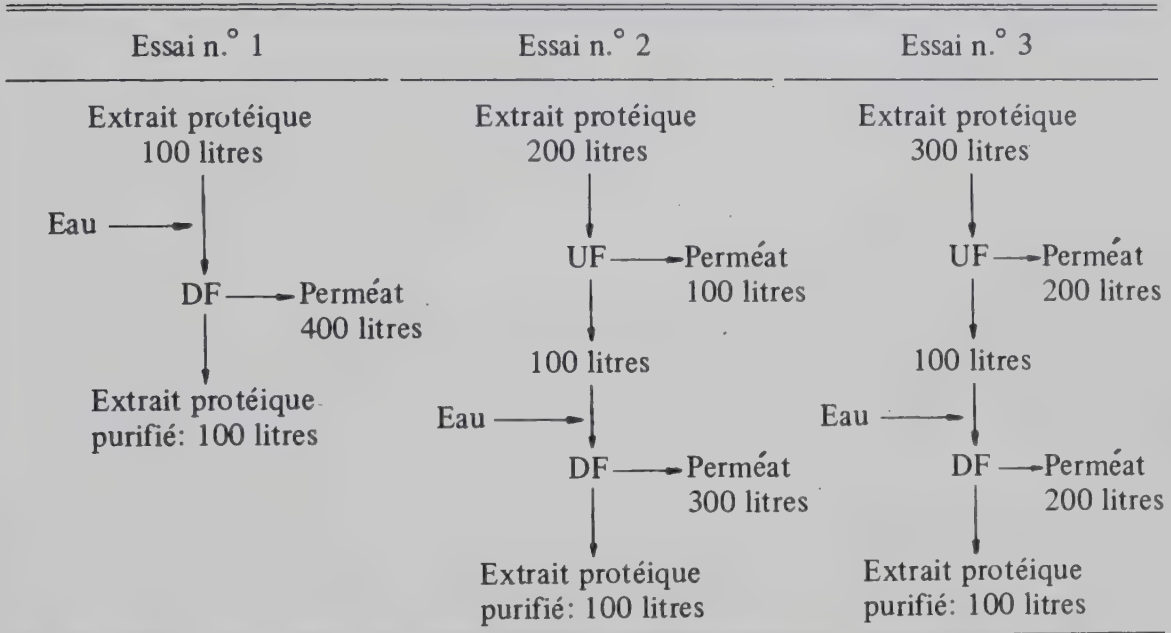
Essai	Type de membrane	Composition	Dimension des pores en Å	Caractéristiques
A	Sartorius SM 113.04	Nitrate de cellulose	8.000	Microfiltration
B	Sartorius SM 113.06	Nitrate de cellulose	4.500	Microfiltration
C	Sartorius SM 117.33	Cellulose régénérée	100-200	Cut-off: 70.000
D	DDS 600	Acétate de cellulose	n.d.	Cut-off: 20.000
E	Sartorius SM 117.36	Cellulose régénérée	50-100	Cut-off: 15.000

Ces essais ont été conduits à partir de 3 litres d'extrait protéique tout en maintenant la concentration constante par recyclage du concentré et du perméat dans le récipient d'alimentation. Les essais ont été effectués à pH 7,3 et à 50° C (±1° C) avec une pression à la sortie de 1 Atm. Toutes les 15 minutes on mesurait la perméabilité (PR = L/m^2, h) jusqu'à sa stabilisation qui était atteinte en 1,5-3,0 heures pour les différentes membranes. Une fois la perméabilité stabilisée on effectuait un prélevement simultané de concentré et de perméat.

Les essais á niveau pilote ont été effectués à 50° C et à pH 8,0 avec des membranes ABCOR type HFA 180 de trois mètres de long et 2,4 cm de diamètre intérieur. La circulation de l'extrait protéique était assurée par une pompe centrifuge capable d'imprimer au liquide une vitesse de 5,1 m/s à l'intérieur des membranes, avec une pression moyenne d'exercice de 1,7 Atm. Les schémas des essais réalisés sont reportés dans le tableau 2. Au cours de chaque essai, à des intervals réguliers on mesurait la perméabilité et on prélevait des échantillons de concentré et de perméat pour effectuer quelques analyses essentielles.

TABLEAU 2

Schémas des essais réalisés à échelle pilote



DF = Diafiltration.
UF = Ultrafiltration.

L'extrait protéique purifié obtenu dans le deuxième essai a été séché par atomisation dans un appareil pilote NIRO ATOMIZER type Minor, avec une température de l'air à l'entrée de 190-195° C et à la sortie de 85-90° C.

Les analyses effectuées sur les échantillons d'extrait protéique et de perméat prélevés au cours des differents essais ainsi que sur les isolats, étaient les suivantes:

pH: déterminé potentiométriquement, à 20° C.

Extrait sec: déterminé par gravimétrie, après séchage a 105° C.

Protéines et azote soluble en acide trichloroacétique (TCA) 12 %.: déterminés par la méthode micro-kjeldhal, d'après les normes AOAC (Anonymous, 1970).

Sucres: déterminés après inversion par la méthode colorimétrique à l'anthrone (Morris, 1958).

Cendres: déterminées par gravimétrie, après incinération à 520° C.

Calcium: par spectrophotométrie d'absorption atomique, avec un appareil Pye Unicam mod. SP 90A.

Phosphore: déterminé d'après le procedé établi par: Int. Dairy Federation (Anonymous, 1967).

Inhibiteurs de trypsine (SBTI): suivant le procédé décrit par Wallace et all. (1971).

Saponines: suivant le procedé décrit par Gestetner et al. (1966).

Activité de l'uréase: déterminée d'après le procedé établi par AACC (Anonymous, 1969).

Indice de Solubilité de l'Azote (NSI) et Indice de Dispersibilité de l'Azote (NDI): déterminés d'après les normes AOCS (Anonymous, 1969).

Lysine disponible: déterminée par la méthode de Carpenter (1960) modifiée par Fabris et Resmini (1968).

Acides aminés: le dosage a été effectué d'après la méthode descrite par Resmini et al. (1969) à l'aide d'un appareil Beckman-Unichrom allié à un integrateur des surfaces des pics Infotromics CRS 110 AB.

La séparation électrophoretique des isolats, des échantillons d'extrait protéique et des pérmeats tel quel et après dyalise, a été effectué d'après Cumming et al. (1973).

Les tests de digestibilité on été conduits sur rats adultes: Trois lots de 50 rats (25 mâles et 25 femelles) choisis par échantillonnage randomisé ont été nourris pendant 10 jours suivant le régime de Hawk et Oser (1954). A partir du 11ème jour et pendant 10 jours dans le régime du deuxième et du troisième lot la poudre de lait écremé a été remplacée par des équivalents protéiques des deux échantillons d'isolat, marqués au préalable à l'oxyde de chrome.

Journellement ont été prelevées les selles de chaque group pour les analyses suivantes:

Extrait sec: déterminé par gravimétrie après séchage a 108° C.

Protéines: déterminées par la méthode de Kjeldhal.

Lipides: par extraction à l'ether éthylique dans un appareil de Soxhlet et séchage à l'étuve.

Cellulose: par la méthode de Weende.

Extractif non azoté: déterminé par difference.

RESULTATS ET DISCUSSION

Le but des essais conduits à niveau de laboratoire était de vérifier les caractéristiques d'un certain nombre de membranes, en vue de l'application que nous étions proposée.

Dans le tableau 3 on a reporté les rétentions p.100[$R\ \% = (1 - C_p/C_c) \times 100$] où C_p et C_c representent la concentration d'un soluté respectivement dans le perméat et dans le concentré, obtenues avec les cinq membranes, pour quelques constituants significatifs. Comme on peut l'observer la rétention des protéines est pratiquement totale, même en employant des membranes à porosité très élevée (essais A et B). Ce phénomène apparement anormal a été déjà mis en évidence par Ariatta-Olivani et Battisti (1974) dans l'ultrafiltration du lait: on doit l'attribuer au phénomène de la concentration de polarisation qui se réalise à l'interface solution-membrane. La rétention de l'azote soluble en acide trichloroacétique augmente avec la diminution de la dimension des pores; la rétention des sucres est très faible. La rétention du calcium et du phosphore semble être constante pour les differentes membranes employées. La rétention de l'uréase est naturellement très élevée étant donné le poids moléculaire de cet enzyme. La rétention des inhibiteurs de trypsine, et notamment des saponines, a été plus élevée que celle qu'on aurait pu

TABLEAU 3

Rétentions p. 100 obtenues avec les différentes membranes employées dans les essais de laboratoire

Essai	Protéines	Azote soluble TCA 12 %	Sucres après inversion	Calcium	Phosphore	SBTI activité	Uréase activité	Saponines
A	97'93	6'3	5'2	84'1	70'9	58'1	94'1	46'3
B	99'06	11'5	5'6	86'4	65'5	64'8	95'0	41'3
C	99'76	16'9	7'3	86'1	70'1	59'4	95'4	68'2
D	99'97	30'1	0'7	74'9	58'1	61'6	96'0	64'1
E	99'80	34'0	–	85'5	72'5	58'8	97'8	80'8

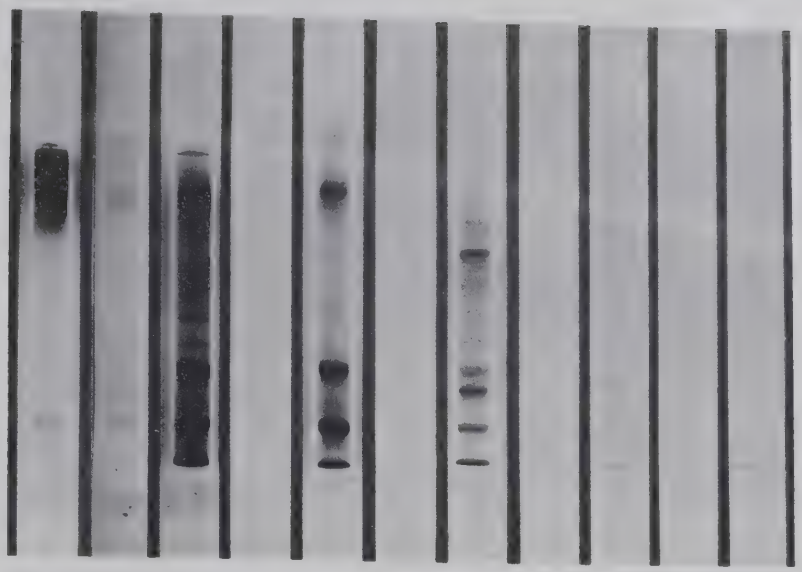


Fig. 1.–Electrophorégramme de (de gauche à droite): extrait protéique lyophilisé; perméat de l'essai A; le même après dyalise; perméat de l'essai B; le même après dyalise; perméat de l'essai C; le même après dyalise; perméat de l'essai D; le même après dyalise; perméat de l'essai E; le même après dyalise.

prévoir d'après le poids moléculaires de ces composés.

Dans la figure 1 on a reporté les électrophorégrammes d'un extrait protéique lyophilisé et des perméats ainsi que des perméats après dyalise obtenus avec les différentes membranes employée. Il est évident qu'avec les membranes les plus compactes (essais D et E) la rétention des protéines est pratiquement totale, tandis qu'avec l'augmentation de la dimension des pores, il se produit une perte, bien que très faible, de molécules protéiques dans le perméat.

D'après ces premiers résultats nous avons pu remarquer qu'il n'existe pas de telles différences qui permettent de choisir avec précision le type de membrane à employer. Ayant toutefois pu observer pour la membrane en acétate de cellulose (essai D) une perméabilité et une rétention de l'azote soluble en acide trichloroacétique plus élevées, nous avons choisi une membrane de ce type pour les essais successifs (Lucisano, 1973).

Les essais que nous avons rapportés ont été conduits à pH 7,3. Afin de rechercher le pH optimum nous avons effectué, avec la membrane choisie et dans les mêmes conditions opératives, quelques essais d'ultrafiltration tout en faisant varier le pH entre 6,3 et 8,5. Cela nous a permis de mettre en evidence une rélation très étroite entre la perméabilité et le pH, comme on peut le voire dans la figure 2. Il est évident qu'il convient de travailler au pH le plus élevé compatible avec la resistance des membranes.

Etant donné les rétentions élevées obtenues dans les esssais précédents, il résulte l'impossibilité d'atteindre par ultrafiltration une purification suffisante des protéines. Pour cette raison dans les essais effectués à échelle pilote nous avons combiné l'ultrafiltration avec la diafiltration. Cette dernière consiste à effectuer une ultrafiltration tout en maintenant constant le volume du liquide à traiter en ajoutant au fur-et-à-mesure de l'eau, en remplacement du perméat eliminé.

Les perméabilités obtenues dans les essais pilote sont reportées dans la figure 3, en fonction des volumes du perméat éliminé p. 100. D'après ces courbes nous avons calculé, pour un procédé discontinu, le temps nécessaire pour atteindre differents niveaux de purification en traitant 100 litres d'extrait protéique avec 1 m^2 de membrane (Peri et al., 1973) (Pompei et al., 1973).

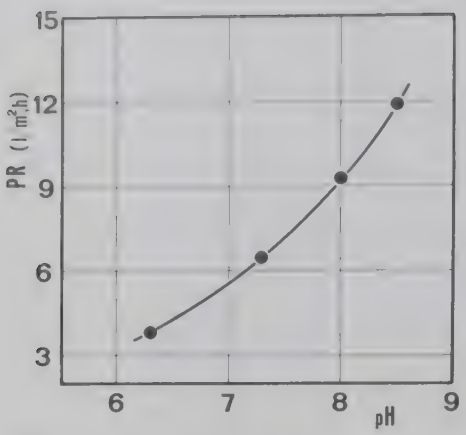


Fig. 2.–Rélation entre la perméabilité et le pH de l'extrait protéique.

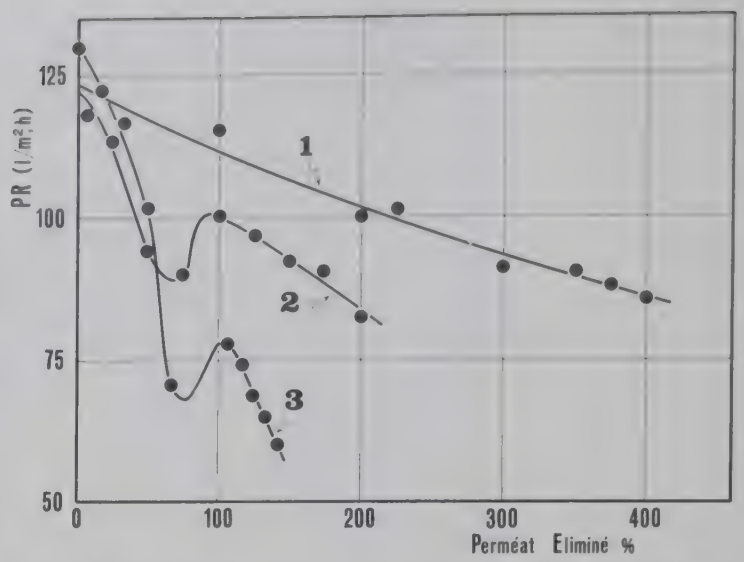


Fig. 3.–Perméabilités obtenues dans les essais pilote, schématisés dans le tableau 2, en fonction des volumes de perméat eliminé p. 10

Dans le graphique de la figure 4 le temps en heures est porté en fonction du rapport de concentration (R) effectué pendant la première phase d'ultratiltration directe. Pour obtenir, p. e., un niveaux de purification de 90 %, exprimé comme rapport protéines/extrait sec, d'après la courbe supérieure on peut constater l'intérêt à concentrer l'extrait au tiers pendant la première phase, avant de commencer la diafiltration. Ce procédé demande, à niveau égale de purification atteinte, un temps inférieure, ou, pour un temps de travail égal, il permet d'employer une surface filtrante inférieure.

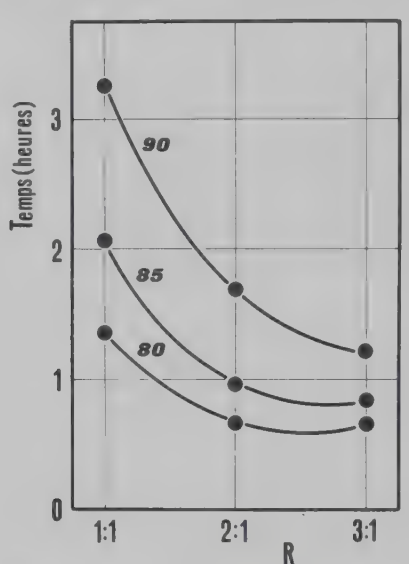


Fig. 4.–Temps (en heures) nécessaire pour obtenir par ultrafiltration et diafiltration différents niveaux de purification des protéines de soja, (90, 85 et 80 %), en fonction du rapport de concentration (R) effectué pendant la première phase d'ultrafiltration directe.

Dans le tableau 4 on compare les résultats analytiques relatifs à l'isolat obtenu d'après la technologie que nous avons employée avec l'un des meilleurs isolat de soja du commerce, obtenu avec la technologie traditionnelle. Les différences les plus importantes concernent une activité plus élevée des inhibiterus de trypsine dans l'isolat que nous avons obtenu, qui presente par contre une teneur plus basse en

TABLEAU 4

Composition analytique de l'isolat obtenu par ultrafiltration et diafiltration et d'un isolat du commerce, obtenu avec la technologie traditionnelle

	Isolat obtenu par UF-DF	Isolat du commerce
pH[1]	7'0	7'0
Humidité, g/100 g.	7'4	4'8
Protéines[2], g/100 g.	83'8	86'6
Protéines/extrait sec, g/100 g.	90'5	91'0
Azote soluble en TCA 12 %[2], g/100 g.	14'6	19'0
Cendres, g/100 g.	5'3	3'8
Lysine disponible, g/100 g de protéines.	3'93	3'74
SBTI (activité)[3]	9'56	4'75
Uréase (activité)[4]	2'18	0'05
Saponines, g/100 g.	3'04	4'44
Indice de Solubilité de l'Azote (NSI).	93'03	67'99
Indice de Dispersibilité de l'Azote (NDI).	93'90	93'00

[1] pH d'une dispersion à 10 % dans l'eau distillée.
[2] Azote X 6'25.
[3] Milligrammes d'inhibiteur par gramme d'isolat.
[4] Exprimée en unité pH, d'après la méthode AACC.

saponines, une solubilité de l'azote nettement superieure et une activité élevée de l'uréase. Cette dernière, qui sérait un indice de mauvaise qualité pour un isolat traditionnel est, dans notre cas, un indice de qualité, puisqu'elle fournit la preuve d'une dénaturation des protéine limitée. Cela est confirmé par les électrophorégrammes de la figure 5, relatifs à un extrait protéique lyophilisé, à l'échantillon d'isolat produit au laboratoire par la nouvelle technologie et à l'isolat du commerce. Comme on peut l'observer, dans ce dernier, la dénaturation plus



Fig. 5.–Electrophorégramme de (de gauche à droite): extrait protéique lyophilisé; isolat obtenu par ultrafiltration et diafiltration; isolat du commerce.

TABLEAU 5

Composition en acides aminés des deux isolats*

	Isolat obtenu par UF-DF	Isolat du commerce
Lysine	6'5	6'1
Histidine	2'6	2'5
Arginine	7'8	7'8
Ac. aspartique	12'0	11'9
Thréonine	4'4	3'7
Serine	5'4	5'5
Ac. glutamique	21'2	20'5
Proline	5'2	5'3
Glycine	4'3	4'0
Alanine	4'0	3'9
Cystine (half)	2'7	2'4
Valine	4'7	4'8
Méthionine	1'1	1'1
Isoléucine	4'9	4'9
Leucine	8'0	7'7
Tyrosine	3'7	3'7
Phenylalanine	5'4	5'4
Tryptophane	–	1'4

* Grammes d'acides aminés par 16 grammes d'azote.

TABLEAU 6

Coefficients de digestibilité des deux isolates et pourcentage de digestibilité (Δ %) par rapport au régime standard

	Groupes de référence		Groupes expérimentaux A				Groupes expérimentaux B			
	Mâles	Femelles	Mâles	Δ %	Femelles	Δ %	Mâles	Δ %	Femelles	Δ %
Protéines	71'43 (±0'36)	72'17 (±0'52)	71'39 (±0'85)	–0'06	72'11 (±0'47)	–0'08	70'26 (±0'41)	–1'64	70'79 (±0'64)	–1'66
Lipides	85.24 (±0'59)	87.20 (±0'86)	85.31 (±0'58)	+0'08	87'08 (±0'73)	–0.14	84'03 (±0'53)	–1'42	85'80 (±0'71)	–1'61
Cellulose.	36.59 (±0'18)	35.15 (±0'27)	36.60 (±0'21)	+0.03	35.20 (±0'20)	+0'14	35'12 (±0'26)	–4'02	34'36 (±0'24)	–2'25
Extractif non azoté	92'89 (±0'61)	90'67 (±0'79)	92'98 (±0'63)	+0'10	90'74 (±0'65)	+0'08	92'07 (±0'77)	–0'88	88'17 (±0'59)	–2'76

A = isolat de soja obtenu par ultrafiltration et diafiltration.
B = isolat de soja du commerce.
Entre parentèse figurent, pour chaque coefficient de digestibilité, les valeurs de la déviation standard.

importante porte à une plus marquée diffusion des bandes. Il est à remarquer en outre que l'électrophorégramme de cet échantillon concerne exclusivement la partie soluble de l'isolat (68 % environ) du fait que la partie la plus dénaturé est complétement insoluble.

Dans le tableau 5 on a reporté l'analyse des acides aminés des deux isolats: il n'est pas possible de mettre en évidence des différences importantes qui, si elles existent, sont favorables à l'isolat obtenu par ultrafiltration.

Les coefficients de digestibilité et les pourcentage de digestibilité par rapport au régime standard sont réportés dans le tableau 6, comme moyenne des dix jours.

On peut remarquer que la substitution dans le régime de Hawk et Oser de la poudre de lait écremé avec l'isolat obtenu par ultrafiltration et diafiltration a très peu d'influence sur la digestibilité des princips nutritifs considerés, à différence de ce qui se produit lors de la substituion avec l'isolat de soja du commerce.

Ces derniers résultats confirment ulterieurement notre hypothèse de départ, c'est à dire que l'ultrafiltration-diafiltration permet d'obtenir une purification du même ordre que celle qu'on peut atteindre avec la technologie traditionnelle mais avec l'avantage d'une dénaturation limitée des protéines, qui se traduit en une meilleure solubilité du produit final avec une meilleure digestibilité.

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Protein isolates from bakers yeast

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ABSTRACT

A practical process has been developed for the commercial production from bakers yeast (*Saccharomyces cerevisiae*) of a 75 % protein concentrate containing less than 2 % RNA and having excellent functional properties. Two other new food ingredients result from the process: bakers yeast glycan, a thickening agent; and bakers yeast extract, a flavoring agent. The development of this process can result in a significant increase in the use of yeast solids in food products because the three new ingredients, bakers yeast protein, glycan, and extract have functional properties superior to inactive dried yeast.

INTRODUCTION

The development of new sources of protein from microorganism for food products has received increased attention during the past few years as the need for ever increasing amounts of protein for both food and feed has been recognized (PAG Bulletin, 1971). Microorganisms to be used as sources of protein should be species known not to produce pathogenic or toxic variants (PAG Bulletin, 1972). The yeast, *Saccharomyces cerevisiae,* meets the criteria having a long history of usage which precludes any question as to safety.

The nutritional value of the *Saccharomyces* strains of yeast has been known for many years (Carter and Phillips, 1944; Sure, 1946; Peppler, 1970; Merrill and Watts, 1955). In food products, the use of dried food yeasts has been limited generally to levels below 5 % because of (1) flavor, (2) lack of functionality in food processing and (3) the level of nucleic acid contained in dried whole yeast. An intake of not more than 2 grams/day of nucleic acid from single cell protein is recommended by the PAG, (1971). Dried whole yeast contains from 5-15 % nucleic acid. For these reasons research was undertaken to develop a commercially feasible process for producing a protein isolate from bakers yeast, *Saccharomyces cerevisiae*, which would be of high protein quality, low in nucleic acid content, bland in flavor and with acceptable functional characteristics for food processing.

MATERIALS AND METHODS

Analytical Procedures

Total solids of samples were determined by drying to constant weight at 105° C. Ash was determined by igniting in a muffle furnace at 550-600° C. until carbonaceous material was absent. Total nitrogen was measured by microkjeldhal method (AOAC, 1970). Total lipid was determined by the polar solvent method of Lee *et. al.*, (1966), and phospholipid by the silicic acid method of Wirth and Magliulo, (1972). The fatty acid distribution was determined by the method of Shehata *et. al.*, (1970). Crude fiber was determined by AOAC, (1970). Amino acid analyses were performed by the column chromatographic method of Spackman *et. al.*, (1958), and by the microbiological methods for methionine, cystine, and tryptophan of Henderson and Snell, (1948).

Nitrogen extractibility is the expression of the amount of protein extracted from the yeast cells defined as the g. of soluble N at pH 9.5 per 100 g of yeast homogenate N.

Nucleic acid was determined by the method of Newell and Robbins, (1973) which is based on the quantitation by liquid chromatography (Uziel *et. al.*, 1968; Burtis, 1970) of purine and pyrimidine nucleosides and bases after treatment of a sample with alkali and alkaline phosphatase.

Protein quality was determined by the PER test (AOAC, 1970) by WARF Institute, Inc.

Bakers Yeast

The bakers yeast used for the preparation of the protein isolates was produced in a commercial bakers yeast plant using the same feeding and fermentation schedules and recovery procedures as for the production of regular commercial bakers yeast. The yeast was grown in highly aerated fermentors by the batch process. The growth substrate was a mixture of clarified and sterilized beet and cane molasses. Ammnonia, phosphoric acid, potassium chloride and magnesium provided sources of nitrogen and phosphorus and minerals for the yeast.

The yeast cells were recovered from the spent broth and washed by centrifugal separation using standard yeast separators. The resulting slurry of yeast cells contained approximately 18-20 % yeast solids.

Results

The process for producing the yeast isolate of low RNA content involves (1) rupturing the bakers yeast cells, (2) solubilizing the protein at pH's at about 9.0-9.5, (3) separating the solubilized protein from the insoluble cell walls by centrifugation, (4) lowering the pH of the protein solution bellow 7.0 and incubating to remove the RNA from the yeast protein resulting in the precipitation of low RNA yeast lipoprotein, (5) recovering the insoluble protein and drying.

Cell Rupture

The concentrated suspension of freshly harvested and washed bakers yeast cells was ruptured by using high pressure homogenizers operating at pressures of 7,000-10,000 PSI. Multiple passes were required to achieve 90 % rupture. The homogenate was cooled through plate coolers between passes.

Protein Solubilization and Cell Wall Separation

The homogenate was diluted to 2-5 % solids at a temperature of 25°-50° C. and the pH raised to 9.0-9.5 with sodium hydroxide. At this pH the proteolytic enzymes are relatively inactive preventing protein hydrolysis. The homogenate which contains the solubilized yeast protein and insoluble cell walls is centrifuged to provide a suspension of yeast cell walls and an alkaline extract of bakers yeast protein. These cell walls, called bakers yeast glycan, can be washed and dried. This yeast fraction has unique thickening properties in aqueous systems.

Removal of RNA and Precipitation of the Protein

The pH of the alkaline extract containing the soluble yeast protein is lowered to about 6.0 with hydrochloric acid and incubated at a temperature of from 30°-65° C. for 30 to 120 minutes. Under these conditions, the yeast endogenous nucleases are activated to hydrolyze the nucleic acids from the yeast protein, thereby reducing the nucleic acid content of the protein. The yeast protein becomes insoluble as the nucleases remove the nucleic acid. About 60 % of the corrected protein content of the yeast is recovered as insoluble bakers yeast protein after the nuclease reaction has been completed. Corrected protein is defined as total nitrogen minus nucleic acid nitrogen times 6.25.

Recovery of the Insoluble Protein

The insoluble protein is recovered by centrifugation, washed and spray dried to provide the product called bakers yeast protein. The thin liquor recovered after removal of the insoluble protein contains all the solubles in the yeast cell including the nucleic acid fractions resulting from the enzymatic activity of the nucleases on the yeast RNA. This fraction, called yeast extract, is concentrated to 70 % solids by vacuum evaporation. If cooked for several hours, this concentrated extract will develop a meat extract flavor.

RESULTS AND DISCUSSION

The fractionation process yields about 60 % of the corrected proteins of the starting yeast solids as a 75 % protein concentrate containing less than 2 % nucleic acids (Table 1). In repeated isolations, the nucleic acid content varied between 0.5 % to a maximum of 2 %. Essentially, all of the lipid is contained in the bakers yeast protein fraction. The lipid fraction contains 54 % neutral lipid and 46 % phospholipid. There are no polyunsaturated C18 : 2 or C18 : 3 fatty acids in the lipid fraction which contributes to the storage stability of bakers yeast protein. The fatty acid moieties of the lipids (Table 2) are composed entirely of C16 : 0, C18 : 0, and C18 : 1.

TABLE 1

Composition of Bakers Yeast Protein

	% Protein (dsb)
Crude protein.	75.2
Corrected protein[1].	74.6
Nucleic acid.	0.7
Ash.	2.2
Lipid.	10.4
Carbohydrate.	12.1
Moisture.	4.4

[1] $\%\ \text{Corrected Protein} = \%\ \text{Crude Protein} - \frac{6.25}{6.13} \times \%\ \text{Nucleic acid.}$

Bakers and brewers yeasts are closely related strains of yeast of the genus *Saccharomyces*. Biological values have been reported for brewers and bakers yeast of from 58 to 77 % (FAO 1970). The determination of the true biological value of yeast protein has always been complicated by the presence of significant levels of nucleic acid nitrogen. The bakers yeast grown for the isolation of bakers yeast protein averaged 7.8 % protein nitrogen and 0.9 % nucleic acid nitrogen. The protein quality (PER) of bakers yeast protein isolate was 2.2 ± 0.1 compared to 1.8 ± 0.1 for the dried bakers yeast (Table 3). In the PER tests the corrected protein previously defined was used to calculate the amount of protein to add to make the 10 % protein level in the diet.

The increase in PER for the bakers yeast protein compared to whole bakers yeast was reflected in the amino acid analyses (Table 4). The levels of cystine and methionine are significantly higher for the bakers yeast protein than for whole bakers yeast. The total sulfur containing amino acids was 2.07 for whole bakers yeast compared to 3.08 for bakers yeast on the protein basis. The first limiting amino acid for bakers yeast protein appears to be methionine. The supplementation of bakers yeast protein with 0.1 % and 0.2 % methionine increased the PER to 2.90

TABLE 2

Classification of Bakers Yeast and Bakers Yeast Protein Lipids

	Bakers yeast	Bakers yeast protein
Lipid, total, % dsb.	6.5	14.0
Phospholipid, % of total lipid.	56	46
Neutral lipid, % of total lipid.	44	54
Classification of fatty acids:		
As % of total fatty acids		
Fatty acid C8:0.	Absent	Absent
C10:0.	Absent	Absent
C12:0.	Trace	Trace
C14:0.	Trace	Trace
C16:0.	18.0	17.9
C16:1.	55.0	52.3
C18:0.	4.0	4.7
C18:1.	23.0	25.1
C18:2.	Absent	Absent
C18:3.	Absent	Absent

TABLE 3

Biological Quality of Bakers Yeast and Bakers Yeast Protein

	Product composition % dsb Corrected protein	RNA	PER[1]
Dried bakers yeast.	47.8	5.6	1.87
Dried bakers yeast.	49.9	5.5	1.80
Dried bakers yeast.	47.9	5.6	1.70
Dried bakers yeast.	50.5	5.3	1.85
Bakers yeast protein.	76.7	0.5	2.11
Bakers yeast protein.	75.8	0.6	2.22
Bakers yeast protein.	77.4	0.6	2.30
Bakers yeast protein 0.1 % dl methionine.			2.90
Bakers yeast protein 0.2 % dl methionine.			3.19

[1] Corrected protein level in the diet was 10 %; PER's calculated to ANRC casein at PER = 2.5.

and 3.19, respectively. All of the other essential amino acids of bakers yeast protein exceed the levels suggested by the FAO (FAO, 1973).

Functional Properties

The bakers yeast protein has an exceptionally bland flavor having the functional properties listed in Table 5. In food applications, bakers yeast protein has been incorporated into beef patties, frankfurters, salisbury steak, chili con carne, cookies, breads, biscuits, candy and dairy type products such as instant breakfasts. The levels vary with the product but up to 30 % hydrated bakers yeast

TABLE 4

Amino acid composition of the protein of whole dried bakers yeast and bakers yeast protein- an isolate from bakers yeast

Amino acid content (grams aa/100 g corrected protein)

	Whole bakers yeast	Bakers yeast protein
Cystine.	0.62	1.16
Methionine.	1.45	1.92
Lysine.	9.06	9.35
Tryptophan.	1.28	1.69
Threonine.	4.91	5.03
Histidine.	2.60	2.86
Valine.	5.74	5.85
Isoleucine.	4.80	5.59
Leucine.	7.42	8.88
Tyrosine.	3.59	4.55
Phenylalanine.	4.28	5.58
Arginine.	5.61	5.91
Serine.	4.83	4.95
Proline.	3.78	4.32
Glycine.	4.95	4.48
Alanine.	6.56	6.16
Aspartic acid.	10.4	11.4[1]
Glutamic acid.	16.9	11.52
% corrected protein (dsb).	49.0	76.8

TABLE 5

Functional properties of bakers yeast protein

Dispersibility
Water absorption
Fat absorption
Emulsification
Gel/coagulation
Thermal extrusion
Fiber formation

protein has been used as a meat extender and up to 10 % in cereal products with no adverse taste effects.

Bakers Yeast Glycan and Bakers Yeast Extract

In any economically feasible process for producing a protein concentrate from a microbial cell, the processing of all the microbial solids into useful food ingredients is essential. In the process for making bakers yeast protein, all the solids are recovered either as bakers yeast protein, bakers yeast glycan or bakers yeast extract.

The bakers yeast glycan is composed of approximately 90 % comminuted cell walls of bakers yeast and about 10 % or less of whole cells. An average analysis is shown in Table 6. Bakers yeast glycan is a carbohydrate polymer composed of

glucose and mannose in a ratio of 3 : 2. The unique functional property of bakers yeast glycan, which has thickening properties in 5-10 % aqueous suspensions, is the fat-like mouthfeel the glycan produces in low-fat or fat-free food products. The glycan has essentially no flavor. The bakers yeast glycan has a caloric value of approximately 0.5 calories per gram.

Bakers yeast extract contains the concentrated yeast solubles resulting from the isolation procedure for producing bakers yeast protein and glycan. The solubles develop meat extract-like flavors when concentrated to about 60 % solids and cooked. The flavor of heat-treated bakers yeast extract is more roast meat-like in flavor than autolysates from bakers yeast. A proximate analysis of bakers yeast extract is shown in Table 7.

TABLE 6

Composition of bakers yeast glycan

	%
Carbohydrate (dsb).	73.0
Fiber (dsb).	14.4
Crude protein (dsb).	11.0
Ash (dsb).	1.0
Lipid (dsb).	0.7
Moisture.	6.2

TABLE 7

Composition of bakers yeast extract

Crude protein (dsb).	47.0
Nucleic acid (dsb).	36.4
Ash (dsb).	22.4
Lipid (dsb).	0.8
Carbohydrate (by difference) (dsb)	29.8
Moisture.	30.0

CONCLUSION

A practical process has been developed to produce from a microbial cell, bakers yeast, a protein isolate high in protein content (75 %), low in RNA (less than 2 %) and of good protein quality (PER = 2.2). The protein isolate from this process is bland in flavor and has valuable functional properties for food processing. The process for making bakers yeast protein yields two other useful food ingredients; bakers yeast glycan, a thickening agent; and bakers yeast extract, a flavoring agent. Essentially, all of the yeast solids are recovered as useful food ingredients essential to an economical process and to eliminate a waste disposal problem.

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Preparation of rapeseed protein isolates

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ABSTRACT

It was demonstrated that, in the precipitation of rapeseed proteins from weakly alkaline aqueous solutions by acidification, the recovery of the proteins could be considerably increased by adding sodium hexametaphosphate or sodium alginate to the solution prior to the addition of acid. This effect most probably depends upon the formation of protein-polymer complexes, which have a very low solubility over a certain pH range.

The precipitates formed in the presence of sodium hexametaphosphate or sodium alginate generally showed a much better processability than those obtained without the use of these polymers. Thus, the dry substance content of the isolates was considerably increased and the product losses during the washing of the isolates were considerably decreased.

Further experiments have shown that the type of effects observed with sodium hexametaphosphate and sodium alginate, can also be obtained with the use of a number of other polyacids.

INTRODUCTION

Rapeseed ranks fifth among the world's oilseeds and is the dominant one grown in Sweden. The seeds contain about 40 % oil and 25 % protein. Rapeseed protein has a very well balanced amino acid composition and is especially rich in sulphur-containing amino acids. These facts explain the current interest in rapeseed as a new source for the manufacture of food-quality protein products, such as protein concentrates and protein isolates.

A protein isolate is usually prepared by first dissolving the proteins by dispersing the proteinaceous raw material in an aqueous alkaline solution. After removal of the insolubles, the dissolved proteins are recovered by precipitation by acid. The precipitate, which is usually washed and dried before use, constitutes the protein isolate. With rapeseed, this simple type of process has been found to give fairly low yields, and to produce wheys with a high protein content. Thus, according to Sosulski and Bakal (1969) and to Girault (1973) as well as to Gillberg and Törnell (1971), a maximum of only 50-55 % of the nitrogen in the raw material could be

recovered in the precipitation step. The fact that the precipitation step gives a low yield with rapeseed is probably a consequence of the complicated protein composition of the seed. Rapeseed is known to contain proteins with widely different molecular weights and iso-electric points. Thus, a large proportion of the proteins, 20-40 %, are strongly basic, with iso-electric points close to 11, and a molecular weight of about 13 000 (Bhatty *et al.*, 1968, Lönnerdal and Janson, 1972). The reminder of the proteins have higher molecular weights and iso-electric points spread out over the range between 4.5 and 8.0.

In our studies on processes for the preparation of protein isolates from rapeseed, we observed that the recovery of protein was considerably improved if the precipitation was conducted in the presence of anionic polymers. Further work showed that by proper choice of polymer dosage and precipitation pH, a quantitative recovery of the dissolved proteins could be achieved. It was also observed that the precipitates formed under these conditions generally had a higher density and gave considerably lower isolate losses during the subsequent washing steps than those formed in the absence of a polymeric precipitation aid.

The aim of this paper is to show the type of effects which can be obtained by adding anionic polymers to the protein solution before the precipitation of the proteins by acid, and to indicate how these effects depend on polymer type, polymer dosage and precipitation pH.

The use of anionic polymers to facilitate the recovery of proteins from wheys and from waste water has previously been reported. In this field reference can be made to the work by Hidalgo and Hansen (1969) and by Hansen (1971) on the use of carboxymethyl cellulose for the precipitation of milk whey proteins, and the work by Spinelli and Koury (1970) on the precipitation of sacroplasmic fish protein by various phosphates.

MATERIALS AND METHODS

Rapeseed meal

The rapeseed meal used in this investigation was prepared by disintegrating defatted rapeseed flakes (obtained from AB Karlshamns Oljefabriker) in a hammer mill (Kamas SKB 200). The meal had a nitrogen content of 7.7. %.

The rapeseed flakes had been prepared in the following way: Seeds from *Brassica napus* (winter) variety, Panter) were crushed in a roller mill. The seed-shells were removed by wind sifting and the oil removed by extraction in a Soxhlet extractor. The extracted flakes were then desolventized in open air (no heating).

Protein dissolution

Rapeseed meal was dispersed in deionized water at room temperature. After adding $MgCl_2$ (57.1 g per kg meal), the pH of the slurry was increased to 8.2 by the addition of aqueous NaOH. The dispersion was stirred for 30 minutes, and the Ph was kept constant by the addition of NaOH. The meal/liquid ratio was 1/20 (w/w). After this treatment, the insolubles were removed by centrifuging the dispersion twice, each time for 10 minutes at 3 100 g in a Wifug X1 centrifuge with swing-out rotor. From the nitrogen content of the solution and the total amount of liquid added, it was calculated that 72 % of the nitrogen in the meal went into solution.

Precipitation

To aliquots of the clarified protein solution sodium alginate (Fluka AG, purum) was added as a 1 % aqueous solution, or sodium hexametaphosphate (BDH, mainly $(NaPO_3)_6$) as a 2 % aqueous solution. After thorough mixing, 0.2 M HCl was added until the predetermined pH was reached. The

dispersion was then centrifuged for 5 minutes at 3 100 g (Wifug X1 with swing-out buckets). The sediment was weighed, and its dry substance content was determined after drying at 85° over night. Protein contents were calculated as 5.5 times the nitrogen content (Tkachuk, 1969). The yield obtained in the precipitation step was calculated as: (total nitrogen in the isolate)/(total nitrogen in the original protein solution).

RESULTS AND DISCUSSION

The results reproduced in Fig. 1 show the effect on the recovery of the dissolved rapessed proteins, produced by the addition of various amounts of sodium hexametaphosphate prior to the precipitation of the proteins by acid. It can be seen that under the experimental conditions used, the yield in the precipitation step could be increased from a per cent recovery of 27 to 78, that is by a factor of almost three.

The extent of increase in yield obtainable by the use of polyphosphate, and the effect of polyphosphate on the detailed shape of the yield versus pH curves is peculiar, and rather sensitive, to the composition of the solution, notably to its electrolyte and protein composition.

However, the results given in Fig. 1 show two features which we have observed in all of the systems studied. Thus, irrespective of the nature of the anionic polymer used, and, within limits, the electrolyte composition, there seems to exist and optimum polymer dosage, which gives the highest achievable yield. In the particular

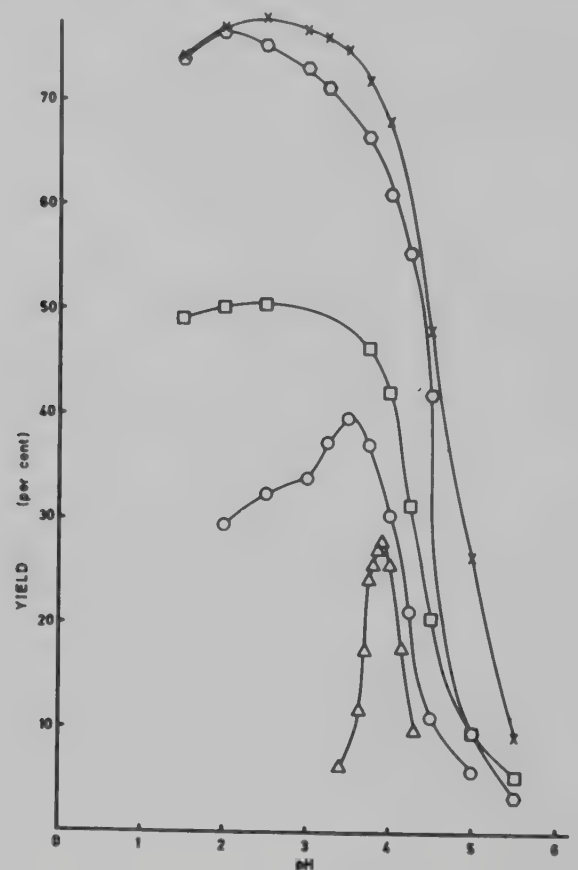


Fig. 1.–The influence of various additions of sodium hexametaphosphate on the nitrogen yield obtained in the precipitation of rapeseed proteins. Sodium hexametaphosphate/protein (N X 5.5) in the original solution (g/g). (△) 0.000, (○) 0.063, (□) 0.258, (X) 0.633, (⬡) 1.007.

system studied in Fig 1, this optimum polymer dosage would correspond to a concentration of polyphosphate close to the next highest concentration tested.

The other common feature is the displacement of the yield maxima when the polymer dosage is increased. From Fig 1 it can be seen that at the extreme concentrations of polyphosphate, this latter effect corresponded to a displacement of the maximum from pH 3.9 to about pH 2.

Both of these features, the existence of an optimum polymer dosage and the displacement of the pH at which the maximum recovery is obtained, can be explained by assuming the precipitate to be a neutral complex formed by reactions between the polymeric anions and the proteins, which at these pH's would carry a net positive charge. Thus, if the polymer dosage is increased, a larger amount of polymeric anions will be bound to the proteins. This gives an increase in the number of negative charges on the complex, and in order to obtain a neutral charge the pH must be decreased. Such a decrease in pH will increase the number of positively charged groups on the protein and possibly decrease the number of negative charges on the protein and the polymeric anions. When the polymer dosage exceeds its optimum value, the amount of negatively charged polymer bound to the proteins may become so high that a complete neutralization of the complex by pH adjustment is no longer possible. This effect would clearly result in a decrease in yield, and it is possible that such an effect played a role in the experiments carried out at the highest polyphosphate concentration (*cf.* Fig 1) As electrostatic forces play a dominant role in the complex formation, any change in the electrolyte concentration is likely to affect the extent of this reaction. It should be understood, however, that pure charge effects are not the only effects involved. Thus, complex formation with the added polymer could bring about large solvation effects, and might even result in the formation of complexes which are water soluble at all pH-values. These solvation effects should be particularly pronounced when a large amount of polymer is added and when the added polymer contains relatively few charged groups.

Fig. 2 gives results which whow that the addition of sodium hexametaphosphate considerably affects the dry substance content of the sediment. A comparison between the results given in Figs. 1 and 2 shows that the dry substance content varied with the concentration of polyphosphate, as did the yield. A high yield thus run parallel with a low water content in the sediment.

At each level of polyphosphate tested, a maximum in the dry substance content was obtained. This maximum occurred at a pH higher than that at which the maximum in yield was obtained. The reason for this is not fully understood.

An increase in the dry substance content, like that demonstrated in Fig. 2, is of great practical importance, as it simplifies the separation of the product from the whey. It was also found that isolates, prepared by precipitation in the presence of a polymeric precipitation aid, could be subjected to thorough washing treatments with very small or without product losses.

The results in Fig. 3 give an example of the effects obtained with a different polymer, sodium alginate. The results are from experiments, carried out at a constant pH of 4.0. As can be seen, sodium alginate would also be an efficient precipitation aid for rapeseed proteins. With this polymer, the maximum recovery was found to be 86 %, which is higher than the maximum recovery (78 %) obtained with the use of hexametaphosphate at a somewhat higher polymer concentration (*cf.* Fig. 1). As a substantial fraction of the nitrogen in rapeseed is non-protein nitrogen, a recovery of 86 % would correspond to an almost quantitative precipitation of the dissolved proteins.

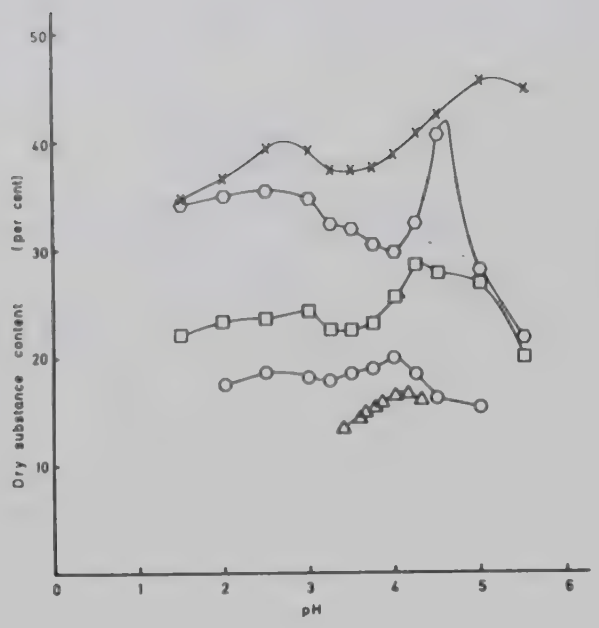


Fig. 2.–The dry substance content of the sediments obtained in the experiments requested in Fig. 1. (Same symbols as in Fig. 1)

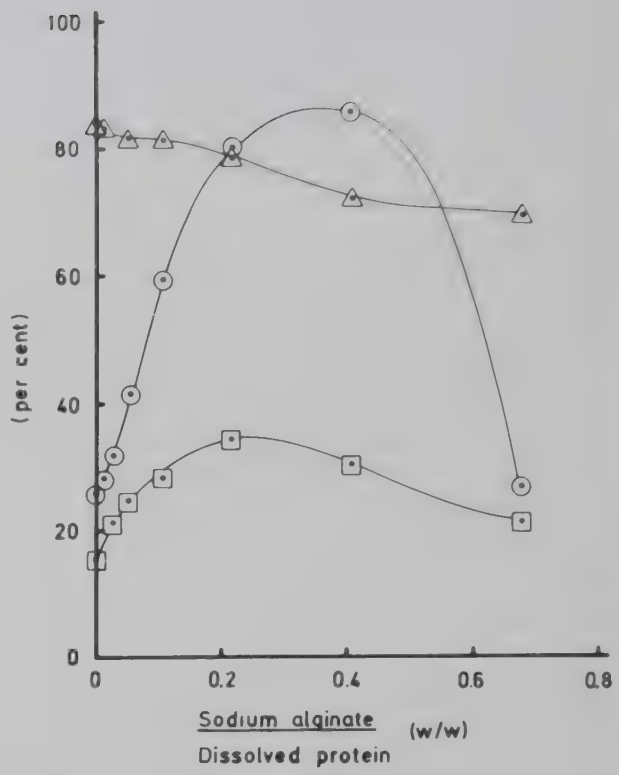


Fig. 3.–The effect of sodium alginate on the nitrogen yield (⊙), the dry substance content of the sediment (⊡), and the protein content of the dried isolate (△) obtained in the precipitation of rapeseed protein at pH 4.0.

A comparison between the yield curve in Fig. 3 and corresponding results taken from Fig. 1, showed that the yield maximum was much sharper with sodium alginate than with polyphosphate. This means that the use of alginate requires a closer control of the polymer dosage than does the use of polyphosphate. This is probably a consequence of the fact that sodim alginate has a lower charge density and a higher molecular weight than hexametaphosphate. This difference in the molecular structure may also offer an explanation of the somewhat lower protein content found in the precipitates obtained with sodium alginate than with sodium hexametaphosphate. From Fig. 3 it can be seen that the isolate obtained under the conditions which gave a maximum in yield contained 72 % protein, whereas the corresponding isolate obtained in experiments with sodium hexametaphosphate at the same pH contained 78 % protein.

The great influence of the electrolyte concentration upon the behaviour of the systems under study can be illustrated by results from experiments with sodium alginate. According to Fig. 3, the maximum yield occurred at a polymer dosage of 0.4 g sodium alginate per g protein. In a similar experimental series, in which no addition of magnesium chloride was made in the dissolution step, the maximum in yield occurred at a polymer addition of only 0.08 g/g protein. Evidently, the electrolyte composition of the solution is one of the most important factors in determining the required polymer dosage and the yield obtained.

Precipitation experiments of the type discussed have been carried out with the use of a number of other negatively charged polymers. These have been applied to solutions of rapeseed proteins prepared under different conditions. Although these experiments have proven that different polymers behave differently, the qualitative behaviour of all of the systems tested was found to be quite similar.

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Simultaneous recovery of protein and oil from groundnuts

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ABSTRACT

A process for the wet milling of groundnuts is described. Processing data are given suitable for large-scale processing. Critical operations were grinding, separation of the fibrous residue and the oil and coagulation of the protein. Optimum recoveries were 91 o/o for the oil and 89 o/o for the protein in isolate form. About 5 o/o of the oil was caught in the isolate. By electron microscopy the emulsion proved to be analogous with cow's milk. A cost price and possible uses for the isolate are given. Criteria for choice of process to obtain protein and oil from groundnuts are discussed. The article includes 4 figures, 2 tables and 22 references.

INTRODUCTION

During the last two decades, several new processes have been developed to concentrate plant proteins for food from several sources. Of these sources, oilseeds are known especially as able to alleviate immediate protein shortage. Together with improvements in conventional oil-milling procedures, these processes aim at recovery of protein with the least possible damage to nutritional and functional quality. Examples of these processes are extraction processes of soybean proteins from carefully defatted soybean flour (A. S. A., 1974), the liquid-cyclone process for glanded cottonseed (Vix et al., 1970) and the wet-milling process for fresh coconut meats (Dendy and Timmins, 1973).

These processes are essentially wet-milling processes using water as well as organic solvents at moderate temperature. The properties of the native proteins are generally best preserved by processing in water, which of course resembles the natural environment in which the protein has been deposited. Another advantage is that no careful removal of solvent is necessary afterwards but only drying or some other technique of preservation.

As outlined in a previous paper (van den Berg, 1972), groundnuts lend themselves very well to wet processing. Especially if the protein concentrate or

isolate be allowed to contain some oil, this type of processing is competitive.

Although several research groups (Chayen and Ashworth, 1953; Smith, 1966) (Sugarman, 1956) (Dangoumau, 1958) (Subrahmanyan et al., 1959; Bhatia et al., 1962, 1966; Eapen et al., 1966; Parpia, 1968), have applied wet-milling principles to groundnuts, recovery generally remained too low to be economic and published processing data were scarce.

But recently Rhee et al. (1972, 1973 a, b, c) published some data on two ways of processing groundnuts wet with rather good yields. One way produced a protein isolate by alkaline extraction and the other yielded a protein concentrate by acid extraction. Both processes could be alternatives to the process described in this paper. Critical processing data and a process-diagram are given, suitable for large-scale processing.

2. MATERIALS AND METHODS

2.1. Groundnuts

Nigerian groundnuts, dehulled, harvest 1969, 1970 and 1972 were used. Skins (testa) and germs were 97 % separated by double passage through a rubber roller mill with different roll-speed (Paul Haase, Hamburg, DBR, no 1863) after slight heating of the skins.

2.2 Pilot plant equipment

stone roller mill, Daub and Verhoeven (Tilburg, Netherlands). No. 053.
cutter, Stephan (Hameln, DBR), UMK 25, 2980 rev./min.
colloid mill, Fryma (Rheinfelden, DBR), rapid LSR 180/61 and Baukastemühle MK 95, 3000 rev./min.
vibration sieve, Seibert Co (Hilversum, Netherlands), 0.075 and 0.15 mm, diam 0,4 m, 50 hertz.
centrifugal decanter, Westfalia (Oelde, DBR), SDB 230, 5500 rev./min.
supercentrifuge, Sharples (Woodchester, UK), No. 1A, 25.000 rev./min. and No. 6, 14.250 rev./min.
basket centrifuge, Heine (Vierssen, DBR), No. 81145, 3.500 rev./min.
belt press, Ensink (Hilversum, Netherlands), length 0.56 m, belt pore size 0.18 mm wrapped with cheese-cloth, speed 127 m/h. The press was originally designed for apple pulp (and made available by the Sprenger Institute, Wageningen).
Wageningen).

2.3 Analysis

Nitrogen: standard Kjeldahl macro-method with SeO as destruction catalyst.
Oil: in fluid samples by HCl-ethanol-light petroleum (b.p. 40-60° C) method as described by Walstra (1961) in solid freeze-dried samples, by direct light petroleum (b.p. 60-80° C) extraction for at least 6 h.
Dry mass: Small samples were dried at 10.4° C untill constant weight which was generally reached after 24 h.
Aflatoxin B 1 was estimated by Mr. G. J. Rundervoort of the Keuringdienst voor Waren (Enschede, Netherlands) by the method of Liem and Beljaars (1970).

All samples were analysed in duplicate and the values given summarize results from several independant process runs.

2.4. Electron microscopy

Electron microscopy was with a transmissión electron microscope, Philips EM 300, and a scanning electron microscope Jeol JSM-U3, (both of the Technical and Physical Engineering Research Service, Wageningen).

3. RESULTS AND DISCUSSION

3.1. Process description

Figure 1 is a process-diagram for the wet-milling of groundnuts. This diagram is the result of detailed study of the separate unit operations and comparison of several types of equipment.

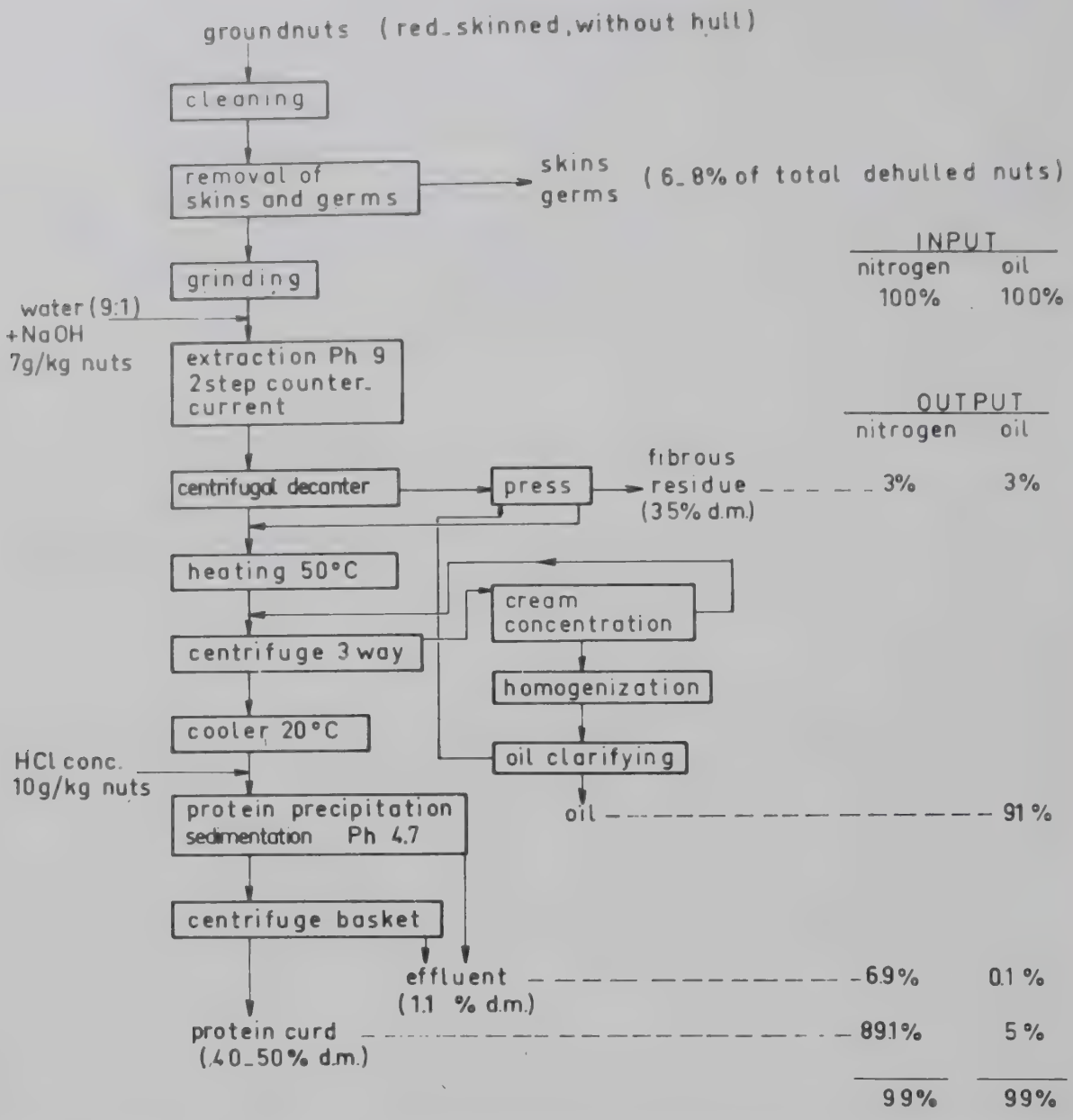


Fig. 1.–Process-diagram of simultaneous recovery of protein and oil from groundnuts by wet milling.

Key operations of the process that can determine recovery seemed to be grinding, separation of the fibrous residue, separation of the oil emulsion and precipitation of the protein. Most operations can be performed in standard equipment of good quality at moderate temperatures. Temperatures below 20° C badly influence extraction of both oil and protein. Temperatures above 45° C produce browning of the protein solution, and temperatures above 80° C reduce protein recovery.

A hygienic dairy-type plant is required for this process. Although pH 9 was antimicrobial, the wet fractions were liable to be spoilt easily. The total count in the effluent after protein precipitation gave data of ca 10^7 microorganisms/ml (determined with standard-plate count agar). Lactic acid bacteria especially are supposed to be very active in the system. A groundnut-suspension stored overnight at room temperature had shifted pH to 4.2.

The nuts have to be free from aflatoxin, because half the original aflatoxin in the nuts was recovered in the protein isolate. Rhee et al. (1973 c) apply chemicals

like H_2O_2 and NaClO to destroy aflatoxin during processing. If the chemicals do not damage the protein the same method can be used in this process after oil separation.

3.2. Grinding

After cleaning and removal of testa and germs, grinding of the nuts can be wet or dry. For oil separation, dry grinding is preferred. Wed grinding (by colloid mill) produced a more stable oil emulsion, suitable if a groundnut milk is required.

Four successive runs through a roller mill with diminishing roller space, for the last run being zero, produced a fine sticky flour giving good extraction. The results of typical screeening of this flour is given in Table 1. Other mills like a mince or pin mill produced too coarse a flour and too much heat. On a large-scale a well designed 5-roller mill will be suitable.

TABLE 1

Particle size distribution of full-fat groundnut flour (350 g) by wet vibration sieving for 2.5 h with a constant flow of tapwater (0.02 l/h). During this operation 88 % of dry matter (solubles, starch and oil) is lost in the effluent

Particle size (mm)	Weight on dry matter basis (% w/w)
> 0.25	7.3
0.21 -0.25	9.0
0.175-0.21	11.0
0.15 -0.175	7.1
0.125-0.15	13.3
0.105-0.125	12.5
0.037-0.105	39.8

Microscopic research showed that the fraction with particles larger than 0.25 mm consisted mainly of unseparated testa particles and lumps of cells, partially still filled with contents. Smaller sizes were well extracted. Particles smaller than 0.1 mm were starch grains and cell-wall fragments. These loose cell-wall particles were difficult to separate because of their shape and small difference in density with their surroundings.

Apart from the outer two layers and some vascular bundles groundnut cotyledons consist, of parenchymatous cells with average diameter of 70-80 m. These cells have to be damaged to release their contents, which are mainly aleurone grains (or protein bodies, 5 - 10 μm), and some starch grains (5 - 10 μm), all surounded by small oil droplets (1 - 2 μm) in a protein film (spherosomes). Figure 2 shows the cellular contents, grains surrounded by oil droplets under scanning electron microscope.

Upon extraction with alkaline water, the protein was solubilized and the oil droplets, originally diam. 1 - 2 μm. were dispersed into emulsion droplets diam. ca 0.1 μm - 25 μm or more. The formation of droplets smaller than 1 μm, important for the oil separation, was found to be difficult to control. The great effect of moisture content of the groundnut, as reported by Eapen et al. (1966), could not be confirmed. Care must be taken to avoid any emulsifying procedures in the process before the oil is separated.

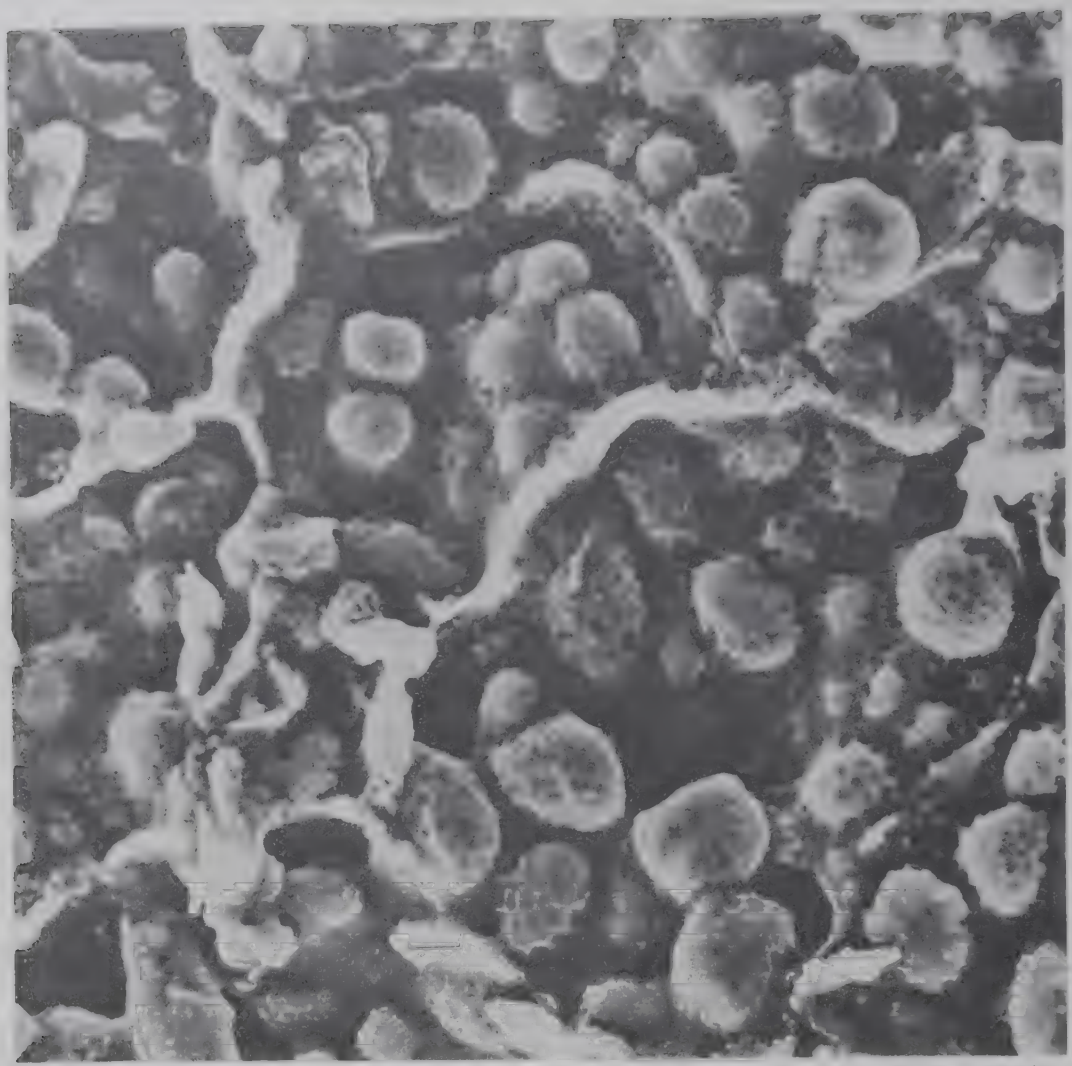


Fig. 2.–Protein bodies and starch grains surrounded by oil droplets in a groundnut cotyledon cell by scanning electron microscopy. The bar represents 5 μm. Surface produced after breaking frozen tissue and fixing with sublimed carbon and gold.

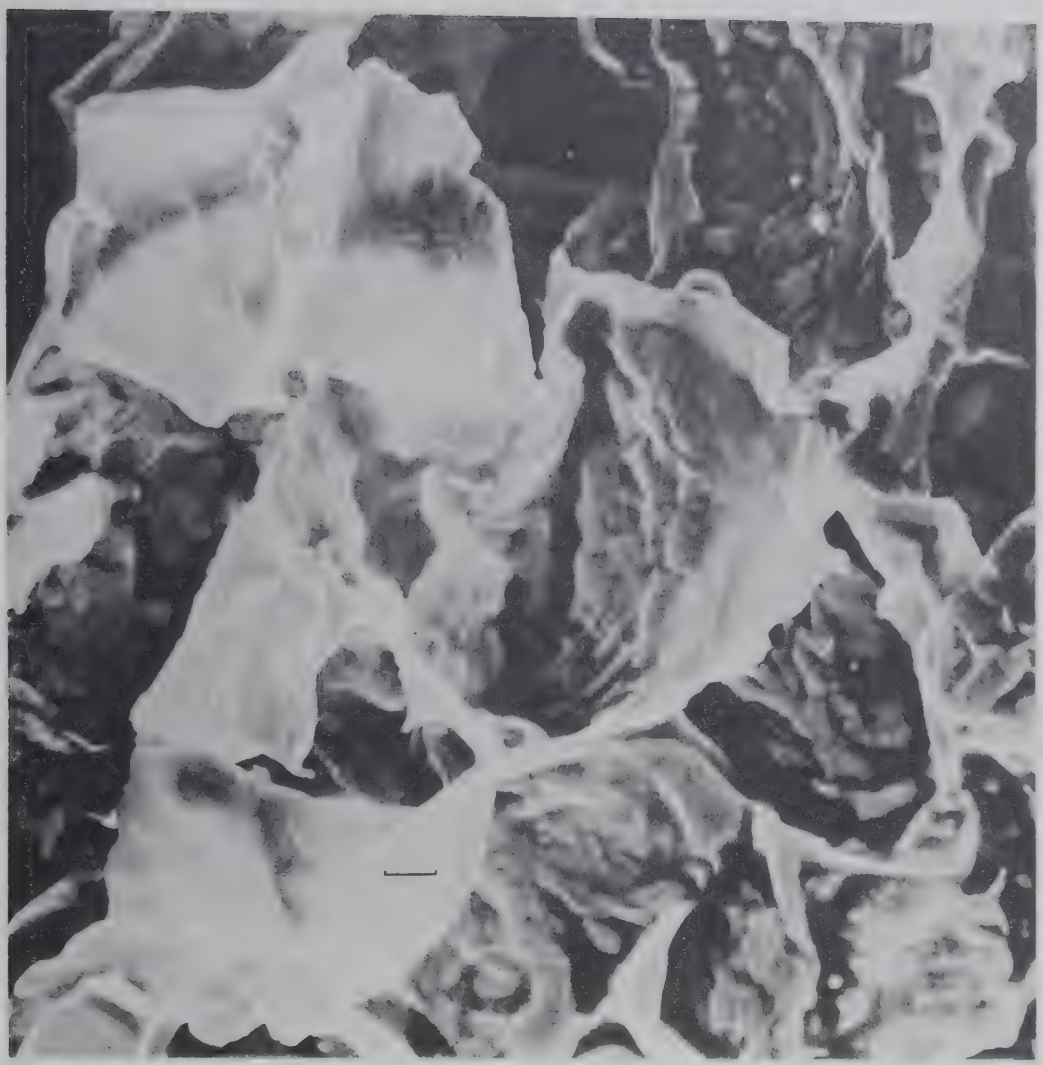
3.3 Extraction

During extraction, constant stirring is necessary to keep the particles in suspension (stirring energy ca 0.2 Watt/litre); 30 min. at pH 9 is sufficient to attain extraction equilibrium. Of several extraction procedures 2 step countercurrent extraction with water-nut ratio of 9 : 1 was found to give optimum results.

3.4. Separation of the residue

The undissolved particles are about 19 % of the suspension volume. On sedimentation, they show a hindered settling behaviour with velocities of 0.4 - 0.6 m/h depending on concentration and temperature, and drag down some of the larger oil droplets. On separation therefore the particles must be caused to move with respect to one other. Vibration sieving with recirculation of the fluid several times and centrifugal decanting gave good results, yielding ca 12 % oil on moisture-free basis in the fibrous residue after 1-step extraction. Filter cakes generally contained 35 % oil. On large-scale, a centrifugal decanter is most suitable. A time of 5 s at 3.200 g is sufficient for clarification.

Further dewatering of the residue (15 - 20 %) dry was carried out by pressing. Dry mass fractions of 35 % were obtained by pressing in thin layers (4 - 5 cm) with a continuous belt press (pressures up to 4.10^6 N/m^2 on the cake). Figure 3 shows a fibrous residue particle after drying. The residue consists mainly of cell walls and is good for cattle feed (ca 10 % protein and 30 % crude fibre).



– Fig. 3. – Ruptured cell walls in a fibrous residue particle by scanning electron microscopy. The bar represents 5 μm. Dried particle fixed with sublimed carbon and gold.

3.5 Oil emulsion

Figure 4 shows the oil emulsion by transmission electron microscopy. This shows that the oil droplets are surrounded by a protein layer ca 10 nm thick (analogous to cow's milk, but with a wider droplet size distribution). Through the high density of this protein (ρ = ca 1,300 kg/m^3), the difference in density between the oil (ρ = 913 kg/m^3 at 25°) and the aqueous phase (ρ = 1,10kg/m^3 at 25° C) disappears for oil droplets diam. ca 0.25 μm. At higher temperatures a better separation is possible because the density difference becomes greater and the viscosity smaller. But, commercially (large-scale) it is not possible to separate oil droplets smaller than ca 0.5 μm by centrifuging. This part, being ca 5 % of the oil phase, is coagulated with the protein. At 50° C a time of ca 1.3 min. at 10.000 g is necessary.

Sludge remaining in the centrifuge contained ca 60 % oil on dry basis, which can be explained by oil droplets smaller than 0.2 μm that are heavier than the water phase.

After separation, the oil is recovered by conventional techniques of oil purification.

3.6. Protein recovery

At the iso-electric point, ranging from pH 4.2 - 4.7, the protein coagulates

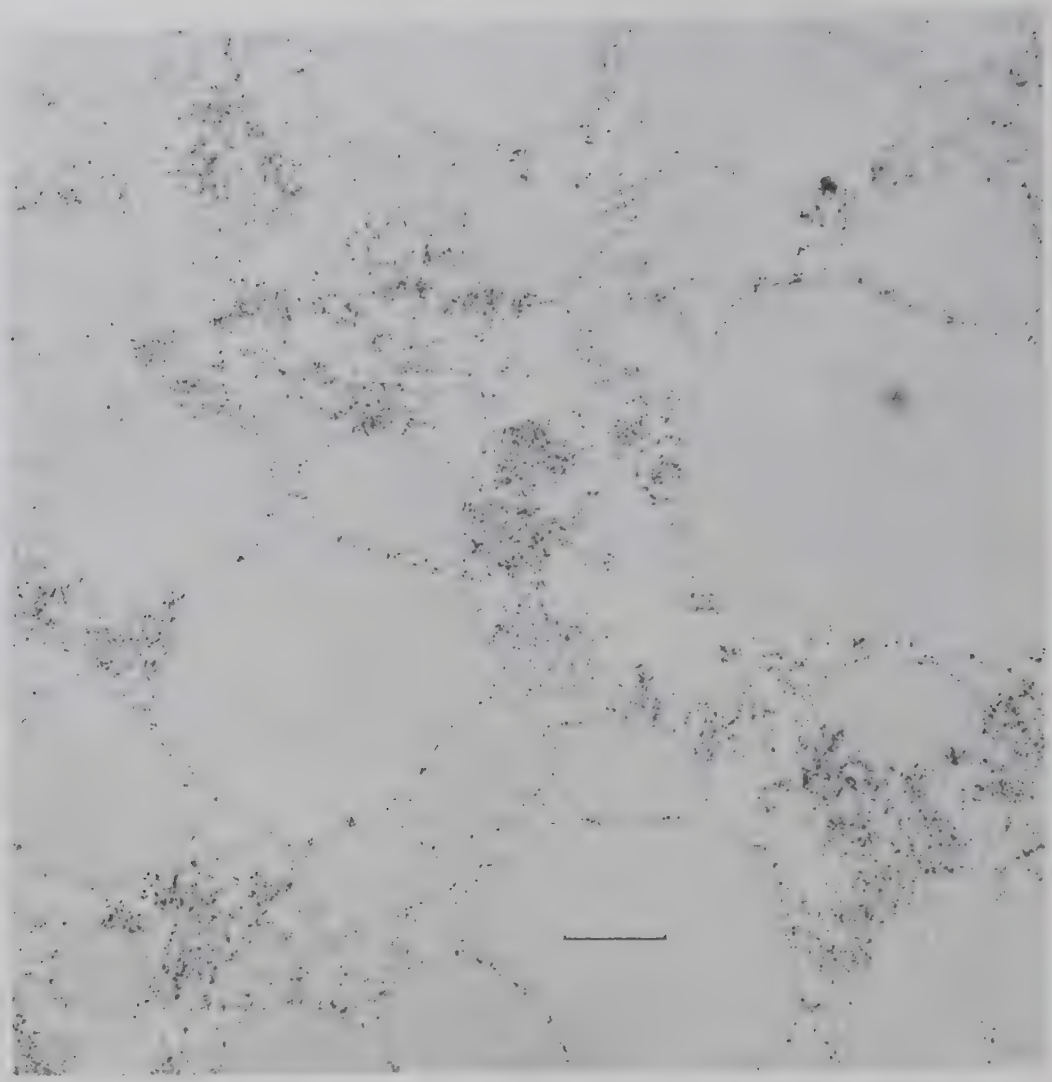


Fig. 4.–Groundnut oil droplets surrounded by protein micelles in emulsion by transmission electron microscopy. The bar represents 0.5 μm. The emulsion was fixed by Salyaev's microcapsule technique as modified by Henstra and Schmidt (1970) and fixed with osmium tetroxide.

into a thick curd, representing 93 % of the dissolved nitrogen. Higher recovery is not possible because the remaining nitrogen is mainly non-protein nitrogen. Only 26 % of the nitrogen in the effluent could precipitate in 5 % aqueous trichloroacetic acid.

After coagulation the curd settles at a rate of 0.12 m/h. Agitation after mixing of the acid lowers the yield by producing small protein particles, which are difficult to separate. Hence the protein curd obtained (ca 20 % of the liquid volume) was further concentrated by a basket centrifuge (1.1 % dry mass in effluent) instead of a centrifugal decanter (2.6 % dry mass in effluent). The curd cannot be filtered. The protein isolate centrifuged for 10 min. at 2.200 g contained 40 % dry mass.

3.7. Uses of the isolate

The protein isolate has many possible food uses (van den Berg, 1972). The quality of the protein isolate in nutritional trials with white wistar rats was found to be the same as that of the original groundnuts.

Groundnut proteins are generally large molecules with a strong association-dissociation tendency. These properties make the protein easy to texturise. Groundnut protein fibres have been reported 25 years ago and were reviewed by Arthur (1953). More detailed work on possible applications of this protein is necessary.

The cost price of this protein isolate produced by a factory of capacity 10.000 tons groundnuts per year under Dutch conditions (1974) was calculated to be about U. S. $ 0.70 per kg dry isolate.

3.8. Choice of process

The recoveries of the process, compared with those of other authors, are shown in Table 2.

It seems possible to recover high percentages of protein and oil by wet processing. Although the oil recovery is somewhat lower than by conventional processing, the recovered protein has a higher yields and a better quality. If the protein required may contain crude fibre and less protein, acid processing as

TABLE 2

Comparison of data of protein and oil recovery from groundnuts

Authors	Extraction ratio water nuts	Process performance	% protein recovered	Composition of protein product (moisture-free basis)		% oil recovered
				% nitrogen	% oil	
Conventional solvent extraction[1] [2]	10-30:1	de-fatted meal as raw material	65-80	16.23	< 1	ca. 98
Wet milling Súbrahmanyan[3] [4]	8.6:1	Batchway single extraction pilot plant	71	13.9-15.2	4.5-12	85
Chayen[5] lypro	12:1	Continuous single extraction commercial scale	78	10.65	33	66
Dangoumau[6]	16:1 (4 × 4:1)	Batchway 4 step extraction lab scale	69	16.5	3.5	92.7
This study	9:1	Batchway 2 step counter-curr. pilot plant	89	14.5-15.7	7-12	91
Rhee[7]	ca 10:1 (6:1 + add. washings)	batchway 3-4 step extraction pilot plant	89	16.3	10.4	86
		[acid processing (ibid)	92.4	12.3	8	89]

[1] J. C. Arthur Jr. (1953).
[2] K.S. Holla (1972).
[3] Subrahmanyan et al. (1959).
[4] D. S. Bhatia et al. (1962).
[5] R. H. Smith (1966).
[6] A. Dangoumau (1958).
[7] K. C. Rhee et al. (1973 c).

proposed by Rhee et al. (1973 a) deserves consideration.

If oil is the most important component, conventional solvent extraction will remain the most economic way of processing ground-nuts. After careful solvent extraction the protein can be isolated with a yield of about 80 % as in present commercial practice, (Holla, 1972). However, when protein especially protein with nativelike functional properties, becomes more important as can be expected, the wet processing of groundnuts will certainly be competitive, especially if the protein isolate may contain some oil.

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Changes in the physical and organoleptic properties of aseptically canned ultra-high-temperature treated ten percent cream during storage

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ABSTRACT

Samples of aseptically canned Ultra-High-Temperature (UHT) processed 10 % cream were stored at 22.8 ± 1°C and 32.2 ± 1°C for 12 months. Gelation or coagulation was not observed in storage samples for up to 12 months. Slight sediment formation was noticed at 9 months at both storage temperatures, but not at 8 months. The organoleptic evaluations revealed no significant changes in flavor and odor during the entire storage period at 22.8 ± 1°C. At 8 months storage, samples at both temperatures were found to be stable as determined by the coffee feathering and alcohol stability tests. The data suggest that sedimentation in the aseptically canned UHT 10 % cream is related to time of storage. The rise of fat globules was more evident in canned cream samples stored at 22.8 ± 1°C than those stored at 32.2 ± 1°C, and also appeared to be related to storage time. Results suggest that aseptically canned UHT 10 % cream is suitable for adding to coffee after a shelf life of 8 months and is suitable for adding to cereal after a shelf life of 12 months.

INTRODUCTION

UHT processing and aseptic canning of dairy products have gained significant importance in recent years due to the long shelf life of sterile dairy products. The literature review by Burton (1969) indicates that processing milk at higher temperatures and shorter holding times results in products having less change in color, flavor, nutritive value, and having a high bacteriological quality. However, milk processed by UHT conditions is reported by Thomé et al. (1964) to have certain changes which are evident only if the sterile milk is more thoroughly tested after being stored for an extended period of time. The flavor is somewhat changed and has a tendency towards a cooked flavor. Some other defects may also develop in the flavor, eg. bitterness. After storing the sterile milk for 14 days, a white sediment

often occurs on the bottom of the containers. After a storage of 4 to 6 months the sterile milk may coagulate and also exhibit whey separation. The product is then unsuitable for human consumption. Unpublished data have shown that 1 : 3 concentrated milk containing 33 % total solids and 6 % fat, when heat treated to 143° C for 8 seconds and aseptically canned, had a shelf life of 3 to 5 months.

There is no literature available on the subject of aseptically canned UHT 10 % cream with a long shelf life. However, Downey et al. (1970) have reported the effects of milk composition and storage conditions on the flavor, physical and whipping characteristics of some UHT whipping creams. They have found that physical stability was lowest in the January to March period, but improved as the storage temperature was decreased. Whipping characteristics were also influenced by seasonal variations in milk composition, but were even more influenced by the storage temperature. A deterioration in whipping performance coincided with gross physical separation after prolonged storage. Lipid oxidation, as measured organoleptically and by chemical means, developed in one of the UHT whipping creams.

The research reported here was conducted to study the changes in the physical and organoleptic properties of UHT 10 % cream during storage and to determine its shelf life.

EXPERIMENTAL

The UHT treated and aseptically canned 10 % cream batches were processed using Cherry-Burrell and Dole aseptic processing equipment. Five pilot plant batches of 10 % cream were made. Each batch was heat treated at 137° C for 8 seconds. The cans were sterilized at 204° C before filling. The samples were then stored at 22.8 ± 1° C and 32.2 ± 1° C for up to 12 months.

Protein stability, cream separation, and organoleptic evaluations were made at intervals of four weeks during the entire storage period. The protein stability was measured by the alcohol and the feathering tests. The alcohol test was conducted by following the descripticon of Samel et al. (1971). One ml portions of aqueous ethanol (96, 83, 72 and 62 %) were added to a series of test tubes, each containing 1 ml of 10 % cream. The tubes were examined for evidence of coagulation both immediately after mixing the contents and after standing for 5 min. The minimum concentration of alcohol which produced coagulation after 5 min. was noted. The feathering test was performed using the method of the Milk Industry Foundation (1964). Nine ml portions of coffee solution, pH 5.0 ± 0.1, were pipetted into 6 test tubes. Increasing amounts of citric acid solution (3.2 g $C_6H_8O_7 \cdot H_2O$ per liter) or sodium citrate solution (11.2 g $Na_3C_6H_5O_7 \cdot 2H_2O$ per liter), from 0.1 to 0.5 ml, were added into 5 of the test tubes. Sufficient distilled water was added to total a 1 ml addition to each tube. In the 6 th tube, 1 ml of distilled water was added into which was placed a thermometer. All test tubes were shaken well, stoppered loosely, and placed in a boiling water bath. When the tube contents reached 96-100° C, one ml of cream was added to each tube and mixed well. After the contents of all tubes had settled, the presence of feathering or curdling was observed. The highest concentration of citric acid which failed to cause feathering was recorded as the feathering value. If feathering value was measured by adding sodium citrate solution, the lowest concentration of sodium citrate at which feathering failed to occur was recorded as the feathering value.

The organoleptic evaluations of UHT 10 % cream during storage were carried out on a large number of coded samples by a trained taste panel, which graded the creams as having normal or cooked flavor, and odor characteristics.

On prolonged storage, a UHT 10 % cream separates into a semi-solid fat layer and low viscosity liquid phases. This separation phenomenon was quantified as grams of cream adhering to the walls after the serum solution was drained from the 10 oz. can.

RESULTS

The results reported are based on the mean value of 5 trials. The physical and organoleptic changes of aseptically canned UHT 10 % cream which occured during storage are presented in Table 1. No coagulation or gelation was observed in the cream samples stored at 22.8 ± 1° C and 32.2 ± 1° C for a period of 12 months. The organoleptic evaluations revealed that the flavor and odor of the 10 % cream samples were normal for the entire one year storage period at 22.8 ± 1° C and that cooked flavor and odor was noticed when the cream samples were stored at 32.2 ± 1° C. It was also observed that the intensity of cooked flavor and odor was directly proportional to the storage time.

The aseptically canned UHT 10 % cream, as measured by the alcohol test, was found to be stable during the 12 month storage period. However, on visual

TABLE 1

Physical and organoleptic changes of aseptically canned UHT 10 % cream during storage

Storage time (days)	Storage temp.	Sedimen-tation	Coagula-tion	Alcohol test	Gelation	Odor	Flavor
30	22.8±1° C	–	–	Stable	–	Normal	Normal
	32.2±1° C	–	–	Stable	–	Normal	Normal
60	22.8±1° C	*	–	Stable	–	Normal	Normal
	32.2±1° C	*	–	Stable	–	Cooked	Cooked
90	22.8±1° C	*	–	Stable	–	Normal	Normal
	32.2±1° C	*	–	Stable	–	Cooked	Cooked
120	22.8±1° C	*	–	Stable	–	Normal	Normal
	32.2±1° C	*	–	Stable	–	Cooked	Cooked
150	22.8±1° C	*	–	Stable	–	Normal	Normal
	32.2±1° C	*	–	Stable	–	Cooked	Cooked
180	22.8±1° C	*	–	Stable	–	Normal	Normal
	32.2±1° C	*	–	Stable	–	Cooked	Cooked
210	22.8±1° C	*	–	Stable	–	Normal	Normal
	32.2±1° C	*	–	Stable	–	Cooked	Cooked
240	22.8±1° C	*	–	Stable	–	Normal	Normal
	32.2±1° C	*	–	Stable	–	Cooked	Cooked
270	22.8±1° C	**	–	Stable	–	Normal	Normal
	32.2±1° C	**	–	Stable	–	Cooked	Cooked
300	22.8±1° C	**	–	Stable	–	Normal	Normal
	32.2±1° C	**	–	Stable	–	Cooked	Cooked
330	22.8±1° C	**	–	Stable	–	Normal	Normal
	32.2±1° C	**	–	Stable	–	Cooked	Cooked
365	22.8±1° C	***	–	Stable	–	Normal	Normal
	32.2±1° C	***	–	Stable	–	Cooked	Cooked

* Trace
** Slight
*** Little

examination slight sedimentation was observed after nine months of storage at both temperatures.

The data presented in Fig. 1 indicate that after eight months of storage at both temperatures, aseptically canned UHT 10 % cream was stable when used in coffee. It was also observed that during the first 5 months of storage the 10 % cream showed lower feathering values at 32.2 ± 1° than at 22.8±1° C. 22.8± 1° C.

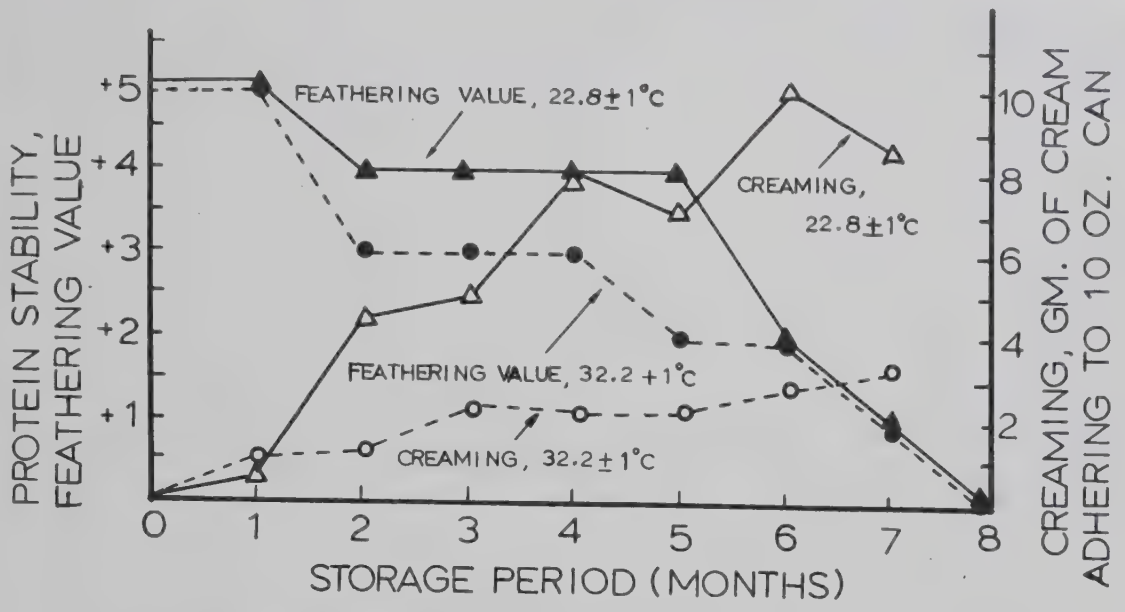


Fig. 1.—Protein stability and creaming of aseptically canned UHT 10 % cream stored at 22.8 ± 1°C and 32.2 ± 1°C.

The results of the investigation of the rising of fat globules in the aseptically canned UHT 10 % cream during the 12 month storage period at 22.8 ± 1° C are presented in Fig. 1. The rising of fat globules was directly proportional to the storage time at both temperatures. The fat globules rose rapidly during the second month of storage at 22.8 ± 1° C. However, the rise of fat globules in the samples stored at 32.2 ± 1° C was slower in comparison with that stored at 22.8±1° C.

DISCUSSION

The organoleptic evaluations revealed that the flavor and odor of the UHT 10 % cream samples were normal for the entire 12 month storage period at 22.8 ± 1° C, and that cooked flavor and odor developed when the cream samples were stored at 32.2 ± 1° C. No oxidized flavor was detected. Lyster (1964) found that all SH groups in milk become free after UHT processing and that on storage for 48 hours at room temperature both the total and free SH groups declined rapidly; on storage at 4° C for 48 hours the decline in SH content was much less. Our panelists reported that the cooked odor and flavor gradually decreased as the storage period increased. This phenomenon might be explained by the fact that the SH groups in milk which developed during processing decreased as the storage period increased. Dill et al. (1962) measured, by titration, free SH groups in milk after steam injection. For a constant holding time the SH titre was at a maximum for a certain processing temperature between 130° and 150° C, and higher temperatures than this gave lower amounts of SH groups. Our results based on sensory evaluation have demonstrated that there was a definite increase in the intensity of cooked flavor in

the UHT 10 % cream during storage at 32.2 ± 1° C.

Our data indicate that the 10 % cream was stable as assessed by the alcohol test during the 12 month storage period at both storage temperatures. The slight sedimentation observed dissappeared on shaking the cans before opening. Coagulation and gelation were not observed in the cream samples stored at 22.8 ± 1° C and 32.2 ± 1° C for a period of 12 months.

The data presented in Fig. 1 indicate that after eight months of storage at both temperatures, aseptically canned UHT 10 % cream was stable when used in coffee. Although the results from the second to the fifth month have revealed that the 10 % cream stored at 22.8 ± 1° C was more stable than the 10 % cream stored at 32.2 ± 1° C, the final feathering values from the sixth to eighth month were identical.

The data presented in Fig. 1 also show that the rising of fat globules in the aseptically canned UHT 10 % cream occurred during the 12 month storage at 22.8 ± 1.° C and 32.2 ± 1° C. The rise of fat globules was directly proportional to the storage time at both temperatures. The fat globules rose rapidly during the second month of storage, and less rapidly thereafter at 22.8 ± 1° C whereas the rising of fat globules in the samples stored at 32.2 ± 1° C was less in comparison. The result of creaming obtained from the present study was opposite to Trout's (1950) results, although he did not have any data for an experiment carried out at 32.2 ± 1° C and 22.8 ± 1° C. Further investigation is suggested.

In conclusion, based on data presented in this study, the aseptically canned UHT 10 % cream processed with Cherry Burrell and Dole equipment has a shelf life of 8 months as coffee cream and 12 months as cereal cream. This property would allow a greater distribution of this product where the availability of fresh 10 % cream is scarce. The data presented also suggest that the UHT processed 10 % cream can be delivered without refrigeration and be warehoused in tropical areas.

ACKNOWLEDGEMENTS

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Rösti, a new industrially prepared potato food product

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ABSTRACT

Rösti is a potato dish prepared according to an old Swiss recipe. Unpeeled potatoes are cooked on the day preceding that of final preparation. By applying this recipe, a form of retrogradation occurs, which gives the product a specific texture and taste.

A description of the developed industrial process (fig. 1) is given.

By this process a product is obtained that equals the home-made Rösti.

The final product is deepfrozen and can be prepared in a short time, direct from deepfreeze.

The analysis of the product:

- moisture content: 72 – 74 %
- dry matter content: 22 – 23 %
- fat content: 3.5 – 4.5 %

The product consists of loose pieces, which can be mixed easily with spices, meat and vegetables during preparation.

INTRODUCTION

Rosa Graf (1964) gives the following recipe of Rösti: "1 kg of potatoes are cooked in their jackets and peeled the next day. On a coarse grater the potatoes are grated. 2 tablespoonfulls of pork-fat and 1 tablespoonfull of butter are allowed to melt in a shallow frying-pan. When melted, grated cuttings are added with a pinch of salt. While carefully mixing together, the cuttings are slightly fried ("browned") over a slow fire. Occasionally, the fried product is turned over, and finally a cake is formed from it. This cake is fried to a golden-brown color on either side, and then the Rösti is placed on a dish. Apart from the potato cuttings, additional ingredients may be used in a Rösti, such as pieces of bacon and cut onions. It is of essential importance that when consuming Rösti, pieces of potatoes are noticed and tasted. Therefore, the potato cuttings must not become so soft during the frying process that they can no longer be distinguished."

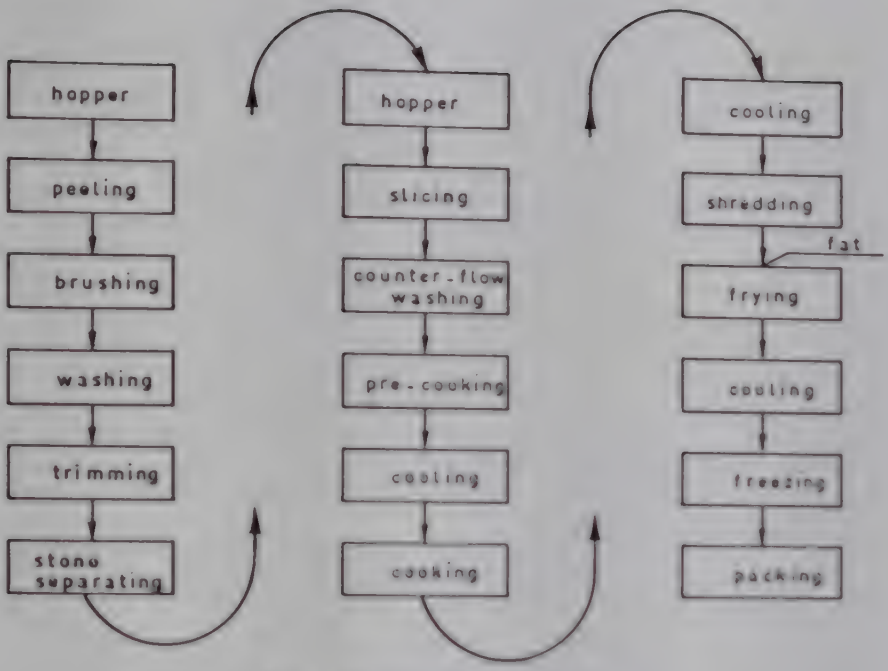


Fig. 1.–Flowsheet of a Rösti plant.

Rösti is most popular in Switzerland, where it is an old and highly appreciated family dish. Although preparations are complicated and time-consuming, Rösti can still be ordered in many Swiss restaurants.

Since a few years, industrially processed Rösti has been on the market. This product is canned and sterilized. Ludwig (1974) tested different makes and reports that these products have a typical "sterilisation" taste, which differs completely from the taste of home-made Rösti prepared according to the abovecited recipe.

The aim of the research and development work carried out in the IBVL (Wageningen) between 1967 and 1972 was to develop an industrial process by which a final product could be obtained that has the same specific characteristics as the home-made Rösti.

Additionally, it was decided to bring the product in deepfrozen form, in order to maintain the quality during several months.

EXPERIMENTAL

Cording et al. (1955) and Cording and Willard (1957) proved the possibility to influence the texture of potato flakes by pre-cooking the raw potato slices at a temperature between 60 and 82° C.

In the British patent no. 1 028 109 (1964) this phenomenon is also referred to. In the process described in this patent, cooling down of the pre-cooked potatoes is effected by passing an airflow over the surfaces of the potatoes while they are still wet with water of the pre-cooking operation.

Starting from the phenomenon referred to above, a pilot plant process was designed including the following stages: pre-cooking potato slices (with a thickness of 15 mm) up to a temperature of 70° C, cooling down with air to a temperature $<25°$ C in the heart of the potato slices, finish-cooking, again cooling with air; shredding the slices into pieces with a lenght of 20-60 mm, a width of 4-6 mm and a thickness of 1-3 mm; frying in a special way and freezing.

When studying the different variations, it became evident that the way of finish-cooking of the potato slices is very important for achieving the good taste and texture of the Rösti.

Best results were obtained with cooking the slices in water or steam for such a time that, if the temperature T in °C measured in the heart of the slices is plotted in a graph against the cooking-time t in minutes, the definite integral to Tdt amounts to 300-900° C min. (British Patent 1265179; 1969).

At evaluating this process in practice, it became clear that intensive washing of the potato pieces was preferable. In the pilot plant therefore a washing machine according to British Patent no. 1340139 (1971) was used.

Further development resulted in the design of a special process and apparatus for continuous frying of potato pieces. This continuously operating fryer of the pilot plant —according to British Patent no. 1343510 (1971)— consists of a plate heated by steam, with the following dimensions: lenght 4.5 m; width 0.6 m; frying-surface 2.7 m^2; the plate is arranged at an angle of 15 degrees. Transport of the potato pieces over the frying-surface is effectuated by a sledge, a vehicle which moves lengthwise over the frying-table. When moving up it scrapes the potato pieces from the frying-plate and with the aid of a small conveyor it throws the potato pieces down again on the plate after having transported them over a distance of about 20 cm downwards. The speed of the sledge and of the conveyor can be adjusted, so that the frying-time of the potato pieces can be controlled. In this frying-system the pieces are turned several times, as a result of which they are fried on all sides and mixed very well. The dwelling-time of the potato pieces on the frying-table amounts to between 6 and 8 minutes.

At the beginning of the frying-table fat is added which is distributed over the full width of the frying-table by means of a perforated tube.

In the pilot plant two methods were applied to finish the product after the frying-operation:

Method 1: after frying, the pieces are formed to a cake and packed in special circular trays; the trays were sealed and frozem.

Method 2: after frying, the pieces were cooled down with the aid of chilled air (temperature: 2° C) and frozen on a belt freezer; after freezing they were packed in plastic pouches of 450 and 600 g.

For final preparation at home the following recipes were drawn up:

Method 1:

melt 1/2 talbespoonfull of margarine (or butter) in a shallow frying-pan
take the deepfrozen Rösti out of the tray and place it in the pan, cover the pan with a lid and fry 9 minutes over a slow fire.
shove the cake out of the pan on a plate, melt again 1/2 tablespoonfull of margarine in the frying-pan.
place the Rösti upside down again in the pan, cover with a lid and fry again 8 minutes over a slow fire.

As salt was added during the industrial process, no further additives were required.

If the Rösti was defrostened before, the total frying-time could be reduced to two times 4 minutes over full fire.

Method 2:

melt 25g of butter or margarine in a shallow frying-pan
strew the frozen pieces in the pan evenly
add salt and other ingredients to taste
cover the pan with a lid and fry 3 minutes over a medium fire
use a pancake-knife to press and form a cake; fry till the undersite is golden-brown
turn the cake —with the aid of a plate— and fry the other side of the Rösti till it is golden-brown too.

RESULTS

At preparing Rösti in the pilot plant according to method 1, the starting-point was to make a Rösti cake that should nicely fit in a normal (shallow) household frying-pan. For this reason salt was added to the potato pieces before frying (7 g per kg). Moreover some Curcuma was added in order to give the interior of the Rösti cake a somewhat yellow color. The finished product was put in a dosing installation, which was measuring a volume dosage. At the end of the installation the product was put in trays which could contain 750 g of potato pieces. The dimensions of the

polystyrene trays were: diameter 220 mm Ø, height 28 mm. After filling, the trays were sealed (see figure 2).

The installation could fill and seal 500 trays per hour.

In winter '70/'71 22.000 Rösti cakes were produced in the pilot plant and frozen.

At regular intervals during production, samples were taken for analysing purposes. Table 1 gives the results, from which it appears that the Rösti cakes were quite homogenious. The sale of this product did not come up to our expectations.

TABLE 1

Rösti, a new industrially prepared potato food product

Potatoes	Rösti			Water evaporation	
% dry matter	% moisture	% fat	% dry matter	% dry matter	Kg/100 Kg Rösti
20.8	69.3	6.7	24.0	0.92	22.08
20.8	68.4	7.8	23.8	0.94	22.37
20.8	66.6	8.9	24.5	1.10	26.95
20.8	66.8	7.9	25.3	1.17	29.60
20.8	66.1	9.8	24.1	1.07	25.79
20.8	67.7	6.6	25.7	1.18	30.33
20.8	66.8	8.3	24.9	1.13	28.14
20.4	66.4	8.6	25.0	1.24	31.00
20.4	66.6	7.9	25.5	1.29	32.90
20.3	66.8	8.3	24.9	1.24	30.88
20.3	68.4	8.0	23.6	1.03	24.31
20.3	67.4	7.8	24.8	1.20	29.76
20.3	66.8	9.2	24.0	1.14	27.36
21.0	66.1	7.8	26.1	1.23	32.10
21.0	67.0	7.3	25.7	1.16	29.81
Average 20.6	66.9	8.1	24.7	1.14	28.22

The retail trade made the following objections:

- the dimensions of the packing were too big for the normal display cases for deepfreeze products
- circular packings are not efficient as far as the loading-rate of a freezer is concerned
- it was difficult to staple the trays in a freezer, because the trays became a little bit bumpy due to freezing
- consumers judged the cake to be too thick and considered the heating-time to be too long
- as the packing became brittle as a result of freezing, the walls of the trays broke off very easy at unpacking.

In order to eliminate the objections of shop-keepers and consumers, it was decided in 1972 to start production of the Rösti according to method 2 (fig. 3). The aim was to prepare a Rösti in such a form that all potato pieces were loose. First, investigations were made on the systems for cooling and freezing of the finished product and on the influence of fats with different solidification points. (Van de Klundert, 1974).

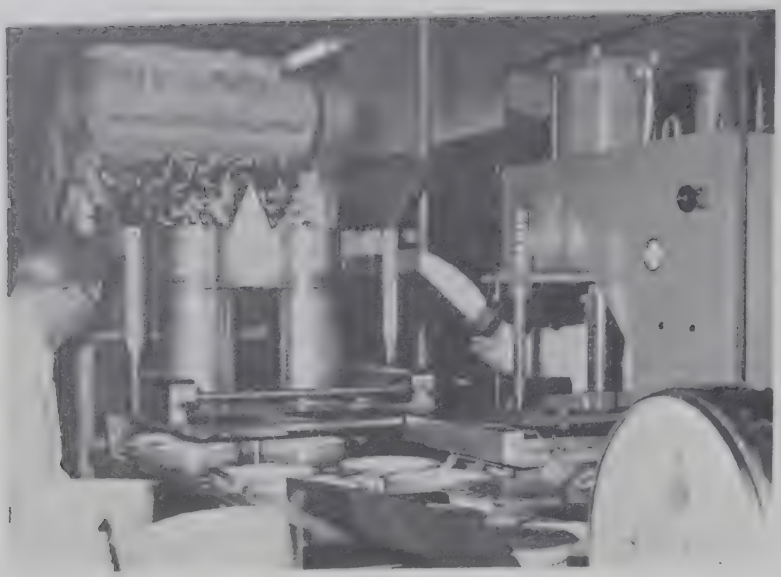


Fig. 2.–Dosing installation (method 1).

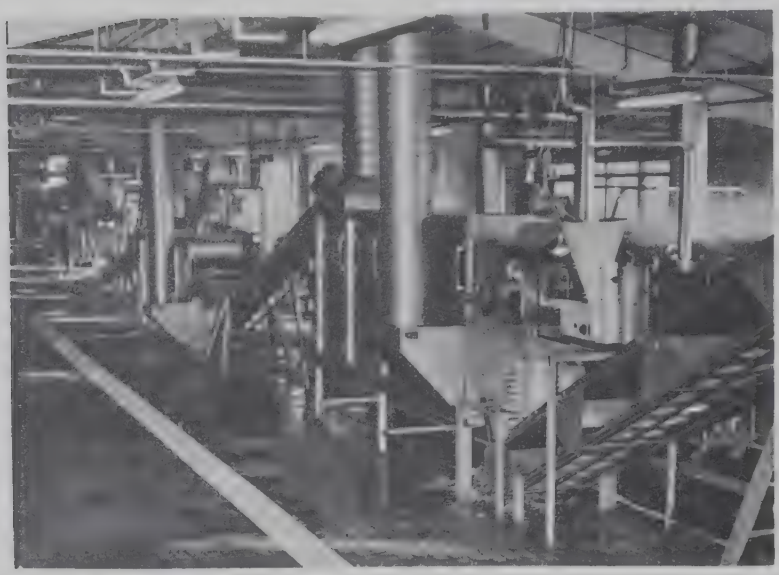


Fig. 3.–Pilot plant for Rösti production.

Best results were obtained with fat with a solidification point of 36° C and with cooling of the pieces in the same way as practice applies at french fry processing. The fried potato pieces were first cooled down on an outside air cooler with 3 conveyor belts, after which they were chilled in a second cooler and finally frozen on a belt freezer. Shortly before sale, the frozen product was packed in 450 g cartons.

After a test market with approx. 35,000 kg of Rösti, it was decided to stop with 450 g packings and to pack the Rösti in plastic pouches with a contents of 600 g.

Till 1974, 155,000 kg of this product was produced in the pilot plant. The deepfrozen potato pieces could be packed with the same machines as other frozen products are packed with.

From harvest 1971, 7000 kg of Rösti was produced in April 1972.

The analysing-results are listed in table 2.

When comparing the data of table 1 with those of table 2, it can be noticed that the shredded Rösti in loose form (table 2) has a much lower fat content (4.4 %

TABLE 2

Rösti, a new industrially prepared potato food product

Potatoes	Rösti			Water evaporation	
% dry matter	% moisture	% fat	% dry matter	% dry matter	Kg/100 Kg Rösti
20.7	71.0	4.8	24.2	0.90	21.78
21.0	69.8	5.1	25.1	0.98	24.60
20.5	69.9	5.8	24.3	1.00	24.30
20.5	73.2	4.1	22.7	0.65	14.76
20.3	73.3	4.1	22.6	0.68	15.37
20.2	73.4	4.2	22.4	0.68	15.23
20.3	73.1	4.3	22.6	0.69	15.59
19.8	74.6	4.1	21.5	0.59	12.69
20.3	73.9	3.8	22.3	0.61	13.60
Average 20.4	72.4	4.4	23.0	0.75	17.54

against 8.1 %), whilst also the water evaporation is lower (0.75 kg/kg of dry matter against 1.14 kg/kg of dry matter).

From harvest 1972, 28,000 kg of Rösti was manufactured in autumn 1972. In table 3 the analysing-data are reviewed. Some samples had a very low fat content (2.0 %, 2,3 % and 2,5 %).

From harvest 1973, 120,000 kg of Rösti were prepared. Table 4 embodies the analysing-data of 40 samples. For this lot of 120 tons of Rösti, potatoes were used

TABLE 3

Rösti, a new industrially prepared potato food product

Potatoes	Rösti			Water evaporation	
% dry matter	% moisture	% fat	% dry matter	% dry matter	Kg/100 Kg Rösti
19.2	72.6	4.3	23.1	1.06	24.49
19.2	72.3	4.8	22.9	1.05	24.05
19.2	72.9	4.6	22.5	0.96	21.60
19.5	76.7	2.0	21.3	0.53	11.29
19.5	75.3	2.8	21.9	0.70	15.33
18.7	75.6	3.1	21.3	0.80	17.04
19.0	74.7	3.3	22.0	0.86	18.92
19.0	75.3	3.5	21.2	0.71	15.05
19.2	73.6	4.5	21.9	0.84	18.40
19.0	74.0	3.1	22.9	1.03	23.59
19.2	74.9	2.3	22.8	0.92	20.98
18.7	73.6	4.5	21.9	0.98	21.46
18.7	75.6	2.5	21.9	0.89	19.49
Average 19.1	74.4	3.5	22.1	0.87	19.36

TABLE 4

Rösti, a new industrially prepared potato food product

Potato	Rösti			Water evaporation	
% dry matter	% moisture	% fat	% dry matter	% dry matter	Kg/100 Kg Rösti
17.7	74.4	3.4	22.2	1.29	28.64
16.8	74.1	3.9	22.0	1.58	34.76
17.7	72.7	4.3	23.0	1.49	34.27
17.7	73.5	4.2	22.3	1.36	30.32
17.7	74.1	4.5	21.4	1.19	25.47
18.2	71.8	4.7	23.5	1.44	33.84
18.5	73.0	4.5	22.5	1.16	26.10
18.2	74.4	3.3	22.3	1.16	25.89
17.7	73.0	3.6	23.4	1.54	36.04
18.5	72.8	4.1	23.1	1.25	28.88
18.2	73.2	4.6	22.2	1.20	26.64
17.7	73.2	5.0	21.8	1.29	28.12
19.0	73.1	4.2	22.7	1.04	23.61
18.2	72.9	4.8	22.3	1.23	24.43
18.7	72.2	4.0	23.8	1.31	31.18
19.0	73.3	3.6	23.1	1.09	25.18
18.5	73.1	4.1	22.8	1.20	27.36
19.0	71.4	4.6	24.0	1.28	30.72
18.2	74.?	4.4	21.4	1.03	22.04
18.2	74.1	4.5	21.4	1.03	22.04
19.2	74.0	4.1	21.9	0.83	18.18
19.5	73.5	4.5	22.0	0.78	17.16
19.2	73.8	4.7	21.5	0.77	16.56
18.0	75.5	4.8	19.7	0.73	14.38
18.2	72.7	4.9	22.4	1.25	28.00
19.5	71.8	4.9	23.3	1.04	24.23
19.0	72.8	4.8	22.4	1.01	22.62
19.2	72.4	4.7	22.9	1.04	23.82
20.2	71.6	5.1	23.3	0.88	20.50
18.7	72.5	4.8	22.7	1.15	26.11
19.2	73.7	3.8	22.5	0.93	20.93
18.0	74.3	4.9	20.8	0.99	20.59
17.3	75.8	4.4	19.8	0.96	19.01
17.5	74.1	4.7	21.2	1.21	25.65
19.2	72.5	5.0	22.5	0.98	22.05
20.2	70.4	4.2	25.4	1.18	29.97
20.0	70.7	4.3	25.0	1.17	29.25
Average 18.5	73.1	4.4	22.4	1.14	25.51

with an average dry matter content of 18.5 %. In this lot of potatoes the highest dry matter content amounted to 20.2 % and the lowest dry matter content to 16.8 %. In spite of this, the lot of Rösti as a whole appeared to be fairly homogenious.
Most of the potatoes used for the Rösti-production were of the variety Bintje.
Depending on size and quality of the raw material, the yield lies between 35 and 55 %.

DISCUSSION

In a pilot plant it has been proved that an industrially prepared Rösti can be produced which has the same characteristics as the home-made Rösti.

The yield of the production process lies between 35 and 55 %. All sizes of potatoes can be used, provided the potato are graded.

The product is marketed in a loose, deepfrozen form, packed in 600 g plastic pouches.

The product has been received favourably by the consumers.

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Effect of experimental variables on the extraction of protein from chilean beans (*phaseolus vulgaris* var. coscorron)

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ABSTRACT

Chilean beans (Phaseolus vulgaris, var. Coscorron) were investigated as a source for the preparation of a protein isolate. The various parameters which influence protein extraction such as pH, contact time, temperature, particle size, ionic strength, and relative amounts of solvent to dry material were investigated. Essentially complete extraction was achieved using the following conditions: pH 9.5-10.0; temperature 23°C (room temperature); time 15 minutes; particle size ≤ 0.4 mm; solvent/ground bean ratio 5:1. About 18 % of the total nitrogen was not precipitated when the pH was lowered to 3.8 (approximate isoelectric point). The provisional amino acid profile shows that the protein isolate is rich in the essential amino acid lysine, i.e., 7.5 % of the protein.

INTRODUCTION

Available studies and recent statistics show that large sectors of the population suffer from serious malnutrition (FAO, 1963). The effect of protein deficiency is particularly serious in children. Such deficiency during the first months of life is the cause of high morbidity and infant mortality indices, and retards physical growth (Errazuriz et al, 1970; Monckeberg et al, 1965). Another disturbing feature is contained in recent reports that protein malnutrition which occurs at certain critical stages when the brain is developing most rapidly is associated with a subsequent irreversible impairment of mental function (Monckeberg et al, 1972).

Raw materials presently being studied in Chile as potential sources of protein isolates include fish, soya, rape and sunflower seeds. Beans are a popular item in the Chilean diet, and its low cost and the good amino acid pattern of its protein would indicate its suitability in the manufacture of isolates.

One of the main difficulties is the extraction of seed proteins. The proteins are

embedded in the seed together with starch and lipids. Extractability of the protein is dependant upon the solvent used and is a function of the conditions of extraction such as pH, salt concentrations, type of salt, temperature, etc. A study of the effect of these factors on protein extraction would be useful from a scientific as well as an industrial standpoint.

The choice of conditions for extraction will be governed by the ultimate objective. Thus, in order to isolate protein in its native state, mild conditions of extraction must be employed. On the other hand, where recovery and economics are important as in industrial preparation of isolates, more severe conditions may have to be chosen.

Thus information about the effect of the experimental variables on extractability of protein will enable protein chemists as well as food processors to use them to their advantage.

The protein isolate must be rich in the essential amino acids, free from toxic or other harmful components and have good functional properties to make it valuable for supplementation to the diet of the vulnerable groups of the population.

The current work is part of a study dealing with the determination, extraction, assessment, and modification of functional properties of isolated edible proteins.

MATERIALS & METHODS

Materials

Chilean bean seeds (Phaseolus vulgaris, var. Coscorron) were obtained directly from a Chilean retailer, about two months after harvest. Normal sun drying had been carried out, and no further treatment previous to this study was performed on the seeds.

Analytical Methods

Sample Preparation. Five hundred g of beans were ground in a Wiley mill to a particle size of 0.4 mm and thoroughly mixed.

Fat. Fat was measured by the standard soxhlet extraction using ether (Pearson, 1973).

Moisture. The moisture content was determined by gravimetric method, after heating the ground samples in an air oven for 3 hours at 105° C.

Soluble Protein. Three g from a bulk crushed sample of beans were shaken in a reciprocating shaker with aliquots of aqueous extractants of different composition and the volumes adjusted to 100 ml. The aqueous mixture was centrifuged at 1,100 x G for 20 min and filtered through Whatman No. 41 filter paper to provide a clear filtrate of soluble protein.

Nitrogen. A recently developed method was used to determine the nitrogen content (Romo et al, 1974). Total or extractable nitrogen was determined by Dye-binding with C. I. Acid Orange 12. The method was adopted because of its simplicity, speed, and high correlation with the standard Kjeldahl procedure.

Amino Acid Analysis. Amino acid analyses were made on acid hydrolysates employing a Technicon automatic amino acid analyzer (TSMI) with on line data logging. Two hundred mg samples were hydrolyzed for 22 hr at 100° C in 40 ml of 6N HCl (Bodwell et al, 1971).

Protein Isolate Preparation. When desired for amino acid analyses, the protein isolate was prepared as follows: To the protein solution after extraction at pH 9.5-10.0 was added 0.1 N HCl to bring the pH to 3.7 (point of minimum solubility). The precipitated mixture was centrifuged at 1,100 x G for 15 min. The precipitate (after washing twice with distilled water), containing the isolate, and the whey proteins were then freeze-dried.

RESULTS & DISCUSSION

Previous research on soya bean meal shows that at least five physical factors affect the extraction of protein with water, namely, size of meal particle,

solvent-meal ratio, temperature, time, and separation of meal residue from suspensoid (Nage et al, 1938). Similar studies on broad beans (Vicia fava) (Flink and Christiansen, 1973) concluded that only the final pH of the solution and bean particle size have influence on the extraction yield.

Extraction of Protein

A large variety of aqueous solvents has been used to extract protein from seeds (Circle, 1950; Lawhon and Cater, 1971; Pant and Tulsiani, 1969). In many studies only the percentage of the total meal protein extracted was reported and few characterisation studies are available on the proteins extracted with different solvents.

Of all the solvents used, water, water plus dilute alkali (pH 7-12) and aqueous solutions of sodium chloride (0.5—2M) are among the most efficient for extracting proteins from soya bean meals (Smith et al, 1966). Because these solvents are also mild, they should yield the proteins in an undenatured state, thus maintaining the original functional properties.

pH of the Extracting Medium

The beans used had received no previous treatment except sun drying, and the proteins were present in the native state. Water plus dilute alkali as extracting medium was chosen.

The extraction data given in Figure 1 indicate that raising the pH to 9.5 or more results in almost complete (>95 %) extraction of protein, and according to earlier classification of proteins (Osborne, 1894), this would correspond mostly to globulins. About 30 % of the protein is soluble at neutral pH. Similar results have been reported for dry navy beans, pea beans, and red kidney beans (Evans and Kerr, 1963; Hang et al, 1970).

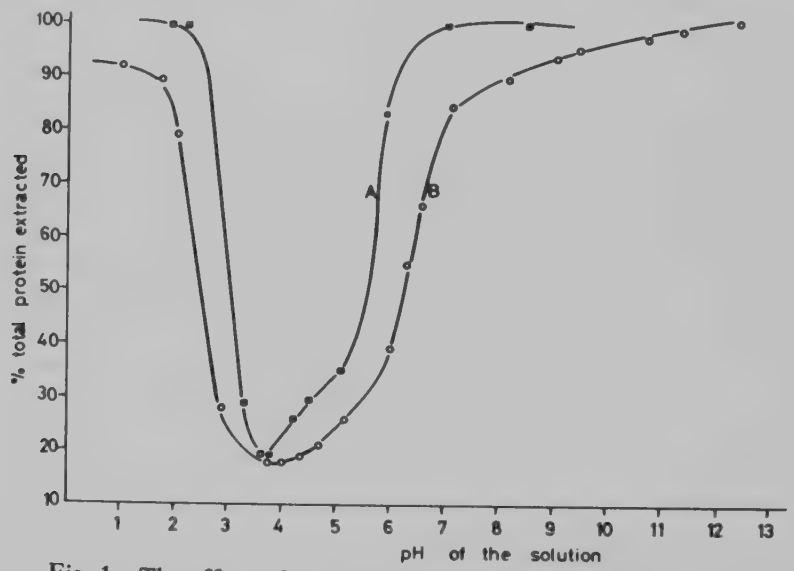


Fig. 1.–The effect of pH on the solubility and extraction of bean protein.

A. . . solubility of protein isolate extracted at pH 9.5 - 10.0
B. . . % protein extracted from the ground bean.

With the addition of acid the protein solubility decreases abruptly and reaches a minimum at a pH range of 3.6-3.8, the isoelectric region; and this property is used for the preparation of the isolate. This region of low protein solubility appears to be at a lower pH than that reported for mung beans, pea beans, and kidney beans, which attained this point at pH 4.0.

Continued addition of acid to reduce further the pH resolubilises the proteins to give solubility values almost as high as those for the alkaline extraction.

pH of Precipitation

After obtaining maximum protein extraction by means of alkali at pH 9.5-10.0, the protein was isolated by precipitation with dilute acid, and the amount of soluble protein remaining was measured at different pH values. The results plotted in Figure 1 show a sharper variation with pH than in the case of extraction.

A very high proportion (>90 %) of the protein isolate remains soluble at neutral pH and this property offers important advantages for the technological utilisation in the preparation of foodstuffs.

Maximum protein precipitation occurs at pH values of 3.6-3.8, a range which is coincident for minimum solubility on both the extraction and precipitation curves. About 17 % of the original bean nitrogen does not precipitate at this pH, and consists mainly of non-protein nitrogen, peptides, and free amino acids. It has been reported that the non-protein nitrogen comprises 10.4 % of the total nitrogen in beans (Powrie, 1961).

Effect of Neutral Salts

Previous reports on the effect of different salts on the solubilisation of nitrogenous constituents from undenatured soya bean meal (Smith et al, 1938) indicated that salt solutions at any concentration dispersed less nitrogenous matter than water. Furthermore, a minimum solubility occurred in the case of univalent cations at 0.1 M, and for divalent cations at about 0.01 M. Other seeds, e. g., wheat, rye, barley, and flaxseed differed from soya bean the sense that solubility did increase as NaCl concentration increased; no minimum solubility was observed.

The behaviour of protein from the tepary bean was similar to that of soya bean, in that a minimum solubility was observed at 0.1 M NaCl concentration. At 0.5 M the solubility remained less than in water (Circle, 1950).

In a comparative study on the nitrogen solubility of mung beans, pea beans, and red kidney beans (Hang et al, 1970) a similar solubility behaviour, followed by an increase in dispersibility was observed. The point minima varied from 0.05 M NaCl for the formed two varieties to 0.025 M for the latter.

With the Chilean beans, a somewhat similar pattern was observed as shown in Figure 2. At low NaCl concentrations a drop in solubility was observed, reaching its minimum at 0.02 M NaCl. Thereafter the solubility increased again, to reach a

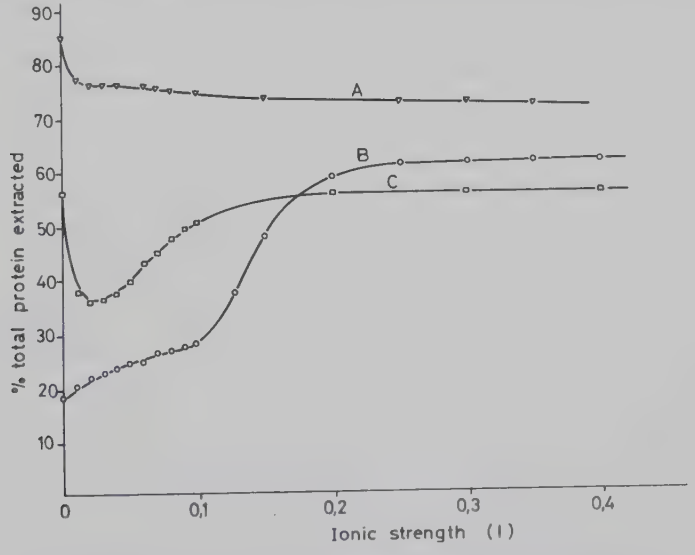


Fig. 2.–The influence of added sodium chloride on the extractability of protein from beans in neutral, alkaline and acidified water.
A = pH 8.0 B = pH 3.7 C = pH 6.5 (distilled water).

maximum at approximately 0.2 M NaCl. Further increase in salt concentration had no effect.

Addition of a divalent cation ($CaCl_2$), showed the same behaviour, i. e. a sharp minimum in nitrogen solubility (35 %) at I values of 0.03 (0.01 M $CaCl_2$) followed by an increase in solubility to a maximum at I = 0.30, and a solubility of 57 % of the total protein.

Chilean beans showed some similarities with soya bean e. g., a high percentage of the nitrogenous material is extracted by water and exceeds that extracted by saline solutions; in the presence of dilute salt solutions sharp minima of protein solubility are observed.

This sensitivity of bean proteins to changes in the ionic environment in aqueous systems can be explained by reversible association-dissociation reactions of the component protein fractions, similar to those suggested for several seed proteins (Koshiyama, 1968 a, b; Putztai, 1965).

Combined Effect of Hydrogen Ion and Neutral Salts

The majority of the seed proteins are considered to be globulins. This class of proteins show minimum solubility in water at the isoelectric point but are rendered soluble by the addition of salts such as sodium chloride or calcium chloride.

The superimposed effect of pH on the dispersion of soya bean protein in salt solution has been studied (Smith and Circle, 1938), and it appears that at low salt concentrations the effect of hydrogen ions is appreciable, and is almost negligible at higher salt concentrations.

For such reasons the effect of neutral salts at pH values near the maximum extractability and isoelectric point of Chilean beans was studied.

As shown in Figure 2, at high pH values, i. e. 8.0, any increase in sodium chloride concentration results in a decrease in the amount of nitrogen extracted from the bean flour. The effect is more marked in the case of $CaCl_2$, as presented in Figure 3.

In the latter case a sharp drop in nitrogen solubility is observed, a minimum being reached at 0.015 M $CaCl_2$ concentration. Subsequently, protein solubility increases but never reaches that of water at that pH.

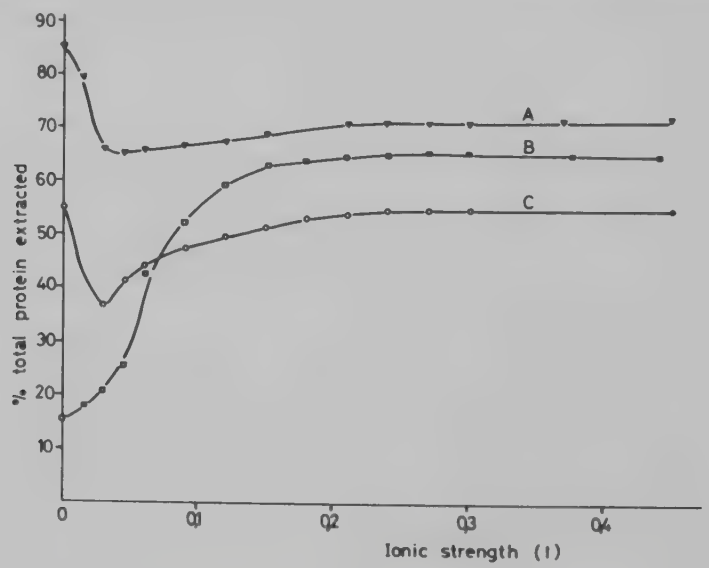


Fig. 3.–The influence of added calcium chloride on the extractability of protein from beans in neutral, alkaline and acidified water.
A = pH 8.0 B = pH 3.7 C = pH 6.5 (distilled water).

When studying the effect of increasing ionic strengths near the isoelectric point the solubility increases with increasing salt concentration. At 0.3 M NaCl, a maximum is reached which exceeds the protein concentration in water extracts of beans.

At increasing $CaCl_2$ concentrations, the effect is even more marked and the maximum is reached at 0.05 molarity, nevertheless the amount of protein solubilized is the same as with NaCl.

The influence of NaCl and $CaCl_2$ on protein solubility at different hydrogen ion concentrations is of some importance when considering possible applications of the isolate. Sodium chloride is a commom ingredient in many food systems, and Ca ions will probably constitute a necessary additive to products such as simulated milks.

Effect of Particle Size

Previous research has indicated that grinding soya bean meal to a fine particle size may increase or reduce protein extraction in water, depending on the nature of the milling action and whether it is accompanied by frictional heating. Dry grinding in a ball mill induced denaturation in soya bean probably by the heat generated in the process (Smith, et al. 1938). This denaturing effect may be avoided by wet grinding, and thus prevent loss in water solubility (Nage, et al, 1938).

The nature of the protein which is resistant to extraction has not been investigated in soya bean or other raw materials but it is possible that part of this protein has been rendered insoluble by reaction with the cations normally occurring in the seed.

It is also suggested (Smith, et al, 1966) that the dispersibility of the protein in a mild solvent, such as water, depends largely on the rupture of the cell structure and membranes within the seed which might prevent contact between the dispersion medium or water and the protein.

Grinding of broad beans to a fine particle size, together with using water of appropriate pH value were found to be the governing factors in protein extractability (Flink & Christiansen, 1973).

A study was made of the influence of particle size on the protein extractability of Chilean beans. Whole beans were ground in a Wiley mill to pass mesh sizes to produce samples of particle size 1.0, 0.78, 0.50, and 0.40 mm.

Each sample was extracted for 15 min with water over a range of pH as described above and the results are given in Figure 4. It is apparent that protein extraction is more complete in the case of the smaller particle size and the extraction curve approaches the protein solubility curve.

Small particle size is therefore shown to be benefitial and is due to shorter diffusion path of the protein inside the particles and to increased cell rupture. The pH of the water remains a factor of major significance.

Effect of Temperature

The effect of temperature on solubilisation of protein from soya bean meal has been studied earlier, and reports indicate that in the temperature range 30 to 90° C maximum extraction occurred at 80° C (Beckel, et al, 1946). In our case, extraction of the bean flour at pH 9.5-10.0 and temperatures of 25, 30, 45, 55, and 60° C had no effect on the yield. As small particle size and appropriate pH values resulted in 95-98 % protein extraction there seems to be no economic basis for extracting at higher temperatures than ambient. Furthermore, although higher temperatures may offer more complete extraction, there is the accompanying risk of heat denaturation of the extracted proteins with consequent loss of valuable functional properties.

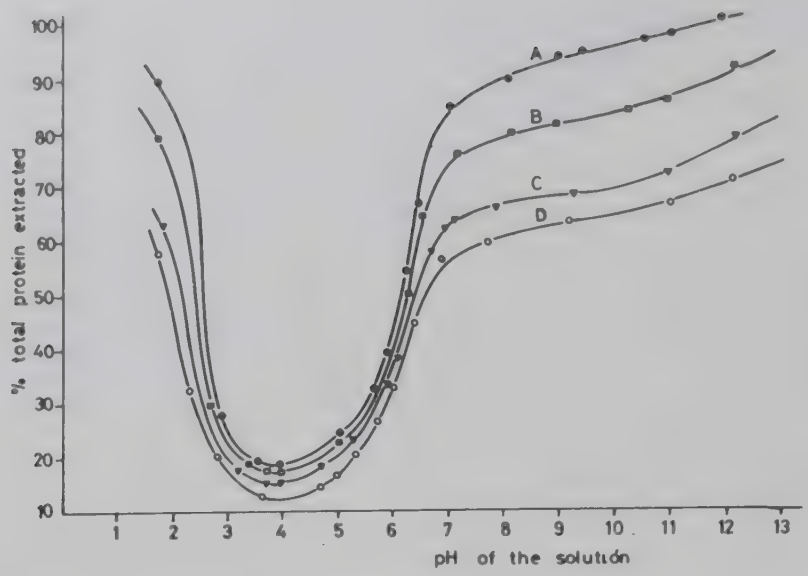


Fig. 4.–The effect of bean particle size on the extractability of protein. A, B, C and D indicate beans ground to pass mesh size 0.4, 0.5, 0.78 and 1.0 mm respectively.

Effect of Time and Solvent: Solids Ratio

The above extraction studies indicate that using 10-15 min contact with water of pH 9.5 or above at ambient temperature maximum extraction is achieved. It is possible that an optimum solvent: solids ratio exists and accordingly ratios of 3 : 1 to 30 : 1 were examined. With ratios in excess of 5 : 1 no significant differences in protein extraction were observed for the different particles sizes. Although the 3 : 1 ratio proved to be equally efficient in extraction, it is difficult to handle during mixing and centrifugation due to the viscosity of the suspension, and for practical purposes a 5 : 1 ratio seems to be most satisfactory.

Amino Acid Analysis

The bea.. flour was extracted in water at pH 9.5 at ambient temperature and centrifuged at 1,100 x G for 15 min. To the soluble extract was added dilute HCl (0.1 N) and the pH adjusted to 3.7. The suspension was centrifuged again at 1,100 x G for 15 min and both the precipitated isolate and soluble whey were freeze-dried.

Amino acid analysis was carried out on the acid hydrolysates of (i) whole beans, (ii) precipitated protein isolate and (iii) whey solubles.

Though the total sulphur amino acids represent a reasonable high percentage of the total amino acids present in the protein isolate i. e. 1.95 % this is considerably less than occurs in animal proteins. The protein isolate should therefore find most beneficial use if supplemented with S-amino acis or animal protein. The lysine content of the protein isolate at 7.64 % is comparable to that of soya bean protein isolates.

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A technology for the fortification of tea with vitamin A

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ABSTRACT

This paper deals with the development of a feasible, low-cost technology for Fortification of Tea with Vitamin A in Pakistan, demonstration and transfer of that technology, and the development of a strategy for implementation of a project to accomplish fortification of most of the tea marketed in that country in order to raise the daily Vitamin A intake of Pakistan's people.

A spray technique, using an air-operated gun and a laboratory pan coater, was developed to apply a specially formulated Vitamin A emulsion to tea material resulting in a concentrate which could be used for dry blending with raw teas prior to sale in Pakistan. Extraction, segregation and storage tests indicated viability of the system and triangular taste testing showed no distinguishable difference from non-fortified tea. It was concluded the concept was viable for introduction into Pakistan and subsequent demonstration and marketing research has indicated the comprehensive plan developed, if implemented, will succeed in supplying important daily intake of Vitamin A to the Pakistani population.

INTRODUCTION

The Government of Pakistan has a great interest in the improvement in the quality of life of its people, not only through agricultural and economic development, but also via a program of supplementation of their indigenous diet to overcome certain apparent deficiencies. This interest prompted a request to the U. S. A. I. D. Mission for assistance in planning and accomplishing programs which could feasibly assist in the amelioration of some of the more debilitating deficencies. An earlier study by the Government of Pakistan (1970) had indicated that among these was an apparent deficiency in Vitamin A in the Pakistan diet, especially among the children and pregnant and lactating women of lower income groups. Also, preliminary investigations by Crowley (1972) had indicated that tea,

the national drink in Pakistan, would be a potential vehicle for use in providing supplemental Vitamin A in the diet. It was felt that it was consumed by most, if not all, of the target population. Further, earlier work by Brooke and Cort (1972) indicated potential success in fortifying leaf tea with Vitamin A applied as a dry powder blended into finished tea or as a diluted water dispersible emulsion sprayed on the hot tea as it emerges from the drying chamber in tea processing plants.

Since tea is imported into Pakistan as finished leaf and/or dust, it was felt that in might be possible to develop a feasible technology for the fortification of tea in Pakistan with Vitamin A. This paper then is a summary of the development of such a technology, the transfer of that technology to Pakistan and the delineation of a marketing strategy for implementation of a program to fortify most or all the tea consumed by the population in Pakistan.

EXPERIMENTAL

The first task of the research team after the completion of library research was to list all the possible ways that finished tea products (as imported) might be fortified. This list was then reviewed by the team with the following constraints: 1) the method or technology should be one that was relatively simple, 2) use supplies and machines that were available in Pakistan (except Vitamin A), and 3) not changing the color, clarity, aroma, appearance or taste of tea or the finished brew. This list of alternative systems was then reduced to the following which seemed to offer the greatest promise; 1) dry blending of tea and powdered Vitamin A, 2) spraying tea material with glycerine to act as an adhesive agent, then applying powdered Vitamin A, 3) spraying tea material with a Vitamin A emulsion-cottonseed oil solution and 4) spraying tea materials with a Vitamin A emulsion - 50 percent sucrose solution.

Prior to testlng any of the systems, a fortification target of 125 IU/g for Vitamin A per cup of brewed tea was established. This target was based upon the assumption that 3 g of dry tea materials would be used to produce one cup (150 ml) of brew, and if a consumer ingested three cups of tea per day, the fortified tea would supply 1125 IU's of Vitamin A, approximately 38 percent of he average RDA (Nutrition Survey of West Pakistan).

Materials

The tea used in the expe~iments was commercially available Ceylon tea of two grades, Broken Orange Pekoe and Dust. Broken Orange Pekoe (B. O. P.) is tea materials from Orange Pekoe leaf grades but smaller particles usually containing tips and buds. The liquors (brew) are generally light or pale in color and usually flavory. Dust are the smallest particles secured in the grading process excluding "fluff and stalk." These teas (dust) are useful for quick brewing and the brews produced have both strength and color, and are used extensively for blending with broken grades.

The Vitamin A materials used were secured from the Hoffman-La Roche Company and are commercially available in the market. The 250-SD powder was a palmitate esther of Vitamin A at 250,000 IU/g. The vitamin emulsion was palmitate esther of Vitamin A in an acacia and water based solution with BHT and dl-alpha tocopherol as anti-oxidants and sodium benzoate as a preservative at 400,000 IU/g.

Test Procedures

The following test systems were designed to provide data which could be applied to the project constrains and assist in the selection of the most feasible technology for fortification: 1) segretation test, 2) shipping test, 3) brewing test, 4) storage test, and 5) taste test.

The segregation test consisted of placing samples of the tea materials (BOP and dust) fortified with Vitamin A in a 4 inch I. D. cylinder. The cylinder was filled (approximately one-half pound) with test material and it was placed in an Eberbach Water Bath Shaker, agitated at a rate of 260 cycles per minute for one hour. Upon completion of the shake period, the cylinder was removed and the contents were divided into their three respective sub-samples - top, middle and bottom one-thirds. Each of the sub-samples were ground on a Wiley Laboratory Mill (30 mesh screen) in preparation for determination of Vitamin A levels. Sufficient numbers of samples for assay were drawn at random from each of the strata and assayed for Vitamin A using Carr-Price procedure[1]. It was assumed that this test procedure

[1]Modified AAOC Carr-Price procedure per conversation with Dr. Cort of Hoffman-La Roche.

would indicate segregation if the levels of Vitamin A in the samples of either of the strata differed significantly from another.

The shipping test was carried out by packing the fortified tea materials in 5 1/2 x 6 inch poly bags and placing them in paper board boxes of 3 1/2 x 3 1/2 x 4 inches. These boxes were then placed into a 200 C flute corrugated shipping carton, sealed and labeled "THIS SIDE UP" and was then shipped by truck for approximately 2500 miles. Upon the return to the research center, the packages were opened and samples drawn from the top, middle and bottom and assayed for Vitamin A to determine if segregation had occurred from "normal" handling and shipping.

Brewing tests were conducted using two methods in preparation of the brew - a steeping and a boiling method. Steeped brew was prepared by adding fortified tea material to water which had been raised to the boiling temperature and removed from the heat source. After five minutes, the brew was placed on a heat source and held at a temperature just under the boiling temperature. In the boiling process, the fortified tea materials are placed in water which is at a rolling boil (100° C) and held at that temperature. The brew was made in a ratio of 3 g of tea per 150 ml water. In addition to taste tests and assay for Vitamin A in the brew, the tea was judged visually for clarity, color and aroma. Also, the spent tea materials were inspected for color, sheen and other properties. In all cases, fortified tea products were judged against unfortified tea materials from the same lot.

An accelerated storage test was established to provide data relative to the shelf-life or stability of the fortified tea under storage conditions. Fifty gram samples of the fortified as well as unfortified control tea materials were placed in 8-ounce glass jars with twist-on covers and placed in storage at 45° C. At the end of 3, 6, 9 and 12 weeks, samples were pulled and assayed for Vitamin A content. The 12-week time frame was equated with one year in storage.

Taste tests were conducted by 8 member panels of experienced tasters. The taste tests were performed on samples with fortification levels from 375 IU to 1200 IU/150 ml. of brewed tea. Samples were tasted against unfortified controls to determine the levels at which Vitamin A was first detectable. Storage samples were evaluated in a similar manner against control samples to determine if differences in taste occurred.

RESULTS

Of the four alternative systems for applying Vitamin A to tea that were tested, the system utilizing the Vitamin A emulsion dispersed in the 50 percent sucrose solution was deemed most appropriate in satisfying the objectives of this study. The two dry blending systems (finely divided Vitamin A powder mixed with tea directly, and finely divided Vitamin A powder mixed with tea after the tea was sprayed with glycerine to act as a sticking agent) were eliminated due to excessive segregation of the Vitamin A as shown in preliminary segregation tests. Although the glycerine and powdered Vitamin A system showed less segregation than the powdered Vitamin A and tea system per se, the addition of glycerine to tea with subsequent application of powdered Vitamin A represented two separate unit processes which would be necessary to arrive at finished product and the emulsion sucrose system required only one unit process.

The system utilizing Vitamin A dispersed in cottonseed oil had the potential for eliminating segregation but brewed tea prepared from tea material containing cottonseed oil was unacceptable because it yielded an oily appearance on the surface of the brew which is deemed unacceptable in the taste tests. In addition, oil when finely divided in an aerosol when used in a production facility would require special attention because of its potential explosivity.

A Batch Process

The system selected for fortification of tea materials was a batch process

yielding a fortified tea concentrate which would require further blending with unfortified tea to achieve the desired level of fortification in the final tea product.

The spray solution was prepared by adding 25 g of Vitamin A emulsion (@400,000 IU/g) to 75 g of a 50 percent sucrose solution to yield a product at 100,000 IU/g., 975 g of tea material was placed in a pan coater turning at 45 rpm and 25 g of the 100,000 IU/g solution was applied with an air spray gun during a period of 45 seconds to evenly distribute the fortificant on the tea. The product called a fortified tea concentrate (a total of 1000 g at 2,500 IU/g) was used as the Vitamin A carrier to be used as one of the inputs for a tea blend. To produce a blend of tea fortified at 125 IU/g rate, 1,900 g of unfortified tea material was placed in a PK Twin-Shell Blender and 100 g of the concentrate added, the machine turned on and the materials blended for one hour. If a target for fortification of 125 IU/g were used and designated as IX, then the following proportions could be used to produce various levels of fortified materials:

1900 g tea and 100 g 20 X concentrate = 2000 g 1 X (125 IU/g) tea
1800 g tea and 200 g 20 X concentrate = 2000 g 2 X (250 IU/g) tea
1700 g tea and 300 g 20 X concentrate = 2000 g 3 X (375 IU/g) tea

These laboratory scale propottions were then used as the basis for plant design and production scale-up.

Test Results

Segregation tests revealed little or no significant migration of the Vitamin A in the fortified tea materials (Table 1) fortified using the selected system or technology.

TABLE 1

Segregation of Vitamin A in leaf and dust tea when applied at various levels as a Vitamin A emulsion dispersed in a 50 percent sucrose solution

Sample	20X level		1X level	
	BOP	Dust	BOP	Dust
	(IU/g)			
Average, blend.	2490	2290	115	110
Top One-Third.	2800	2250	130	110
Middle One-Third.	2800	2200	140	90
Bottom One-Third.	2740	2250	120	80

*See text for description of test.

Shipping tests also indicated no apparent segregation of Vitamin A (Table 2) in the tea materials fortified using the emulsion-sucrose spray system.

The results of the brewing tests (Tables 3 and 4) indicated that generally a sizable proportion of the Vitamin A was available in brewed tea and that the release was relatively rapid — usually within the 1-5 minute range for boiling and even more rapid and constant using the steep process. Visual examination of the brew as well as the spent tea materials showed that no apparent difference in color, clarity, aroma or sheen existed between fortified and unfortified teas.

Several taste tests were conducted to determine if fortified tea brew was different or "odd" from brews prepared with unfortified teas. At no time at the various lower levels 1X, 2X and 3X were data secured that indicated panelists were able to detect tea fortified with Vitamin A. Also, taste tests were conducted using

TABLE 2

The effect of shipping on the stratification of Vitamin A applied to leaf and dust tea in a Vitamin A emulsion dispersed in a 50 percent sucrose solution

Sample	20X level		1X level	
	BOP	Dust	BOP	Dust
	(IU/g)			
Average, blend.	2500	2500	125	125
Top One-Third.	2700	2340	122	108
Middle One-Third.	2700	2340	122	125
Bottom One-Third.	2250	2340	122	125

TABLE 3

The effect of fortification level, brewing time and form of tea on the extractability of Vitamin A from fortified tea

Fortification level	Brewing time in minutes	Tea			
		BOP		Dust	
		Assay (IU/g)	Target[1] %	Assay (IU/g)	Target[1] %
1X,375 IU/cup.	5	353	94.1	285	76.0
	30	413	110.1	353	94.1
	60	690	184.0	330	88.0
2X,750 IU/cup.	5	787	104.9	563	74.9
	30	870	116.0	637	84.9
	60	885	118.0	630	84.0
3X,1125 IU/cup.	5	1050	93.3	825	73.0
	30	1050	93.3	900	80.0
	60	1050	93.3	938	83.4

[1] Actual assay results can be interpreted as being ± 10 %, the established Carr-Price error rate, and for those readings in excess of 100.0 percent of target there was possible interference in colorimeter readings for releases of organic compounds from tea.

tea fortified at 1125 IU/cup and in a triangle test procedure using eight panelists, the highly fortified tea was found not to be statistically different in taste when tested against "normal" or unfortified brews.

The storage results (Table 5) demonstrated loss in the Vitamin A content of the fortified tea materials in storage. The BOP fortified tea materials lost approximately 40 percent of the Vitamin A during the 12 week test while the dust lost approximately 54 percent of the Vitamin A. The average for loss of Vitamin A for the aggregate materials in storage was (sum of assay values at end of 12 weeks divided by sum at 0 week) about 47 percent. From this, one could assume that fortified tea in storage for one year would lose about one-half the Vitamin A content. Thus, any program developed to furnish supplemental Vitamin A through the

consumption of brewed tea will require the adjustment of the initial fortification levels for Vitamin A to account for the loss of Vitamin A which would be experienced in storage over time. It was recommended that the implementation level for Vitamin A in Pakistan be double the target level to account for such losses in Vitamin A from the time of preparation to consumption. The target was 125 IU/g and consequently, the tea was to be fortified at 250 IU/g.

TABLE 4

The effect of preparation method and time on the extractability of Vitamin A from fortified tea

Time sample Drawn, minutes	Assay by preparation method		% 375 IU/target	
	Boil	Steep	Boil	Steep
	(IU/g)			
1	–	353	–	94.1
2	323	353	86.1	94.1
3	330	338	88.0	90.1
4	–	353	–	94.1
5	353	353	94.1	94.1
10	338	353	90.1	94.1
15	356	–	94.9	–
20	413	–	–	–

TABLE 5

The effect of accelerated storage at 45 degree centigrade on Vitamin A retention and Vitamin A fortified leaf and dust teas

Type of tea	Storage time in weeks				
	Initial (0)	3	6	9	12
	(IU vit. A/g)				
BOP.	117	82	77	61	50
BOP.	270	200	140	92	104
BOP.	378	280	256	168	176
BOP.	2250	–	1760	1241	1480
Dust.	104	90	72	78	38
Dust.	274	182	152	110	125
Dust.	360	260	200	160	141
Dust.	2500	–	1800	1400	1200

DISCUSSION

In the transfer of the technology, the research team engineers designed and constructed a demonstration device (Figure 1) which was delivered to Pakistan and used to demonstrate the simplicity of the system devised to accomplish the

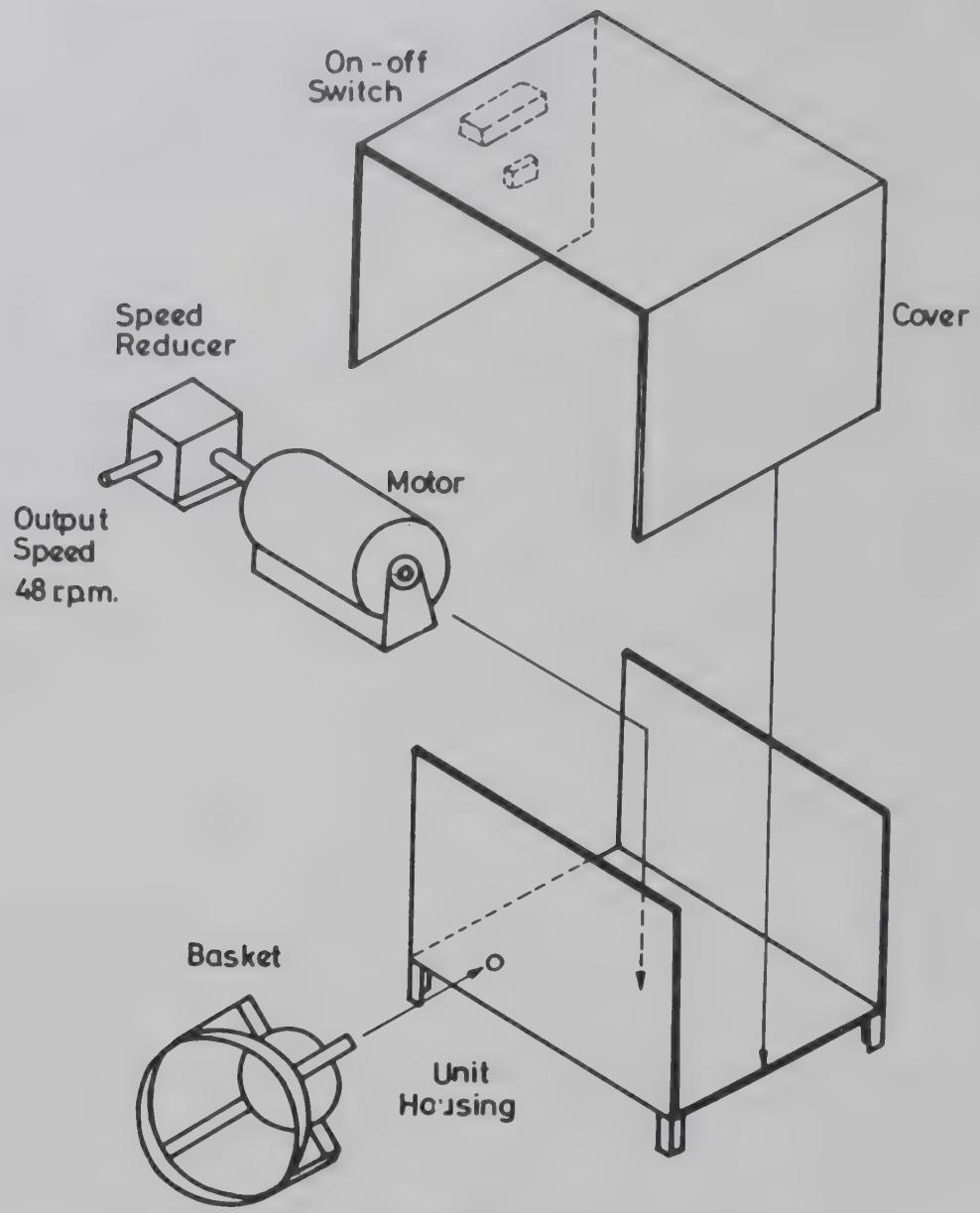


Fig. 1.–Tea fortification demonstration unit.

fortification of tea with Vitamin A. A food processing engineer from the research team conducted demonstrations using the pilot model before interested officials in the Government of Pakistan, the U. S. A. I. D. Mission team, tea blenders and merchants and other interested parties throughout the country (Islamabad, Karachi, Rawalpindi, and Peshawar).

Detailed process descriptions and schematic drawings were prepared for plants with production capabilities of 500, 1500 and 4500 thousand pounds of 20 X concentrate per year. In addition, another plant was presented with the capability of producing 1,500,000 pounds of concentrate and fortifying 30 million pounds of fortified tea per year. Equipment lists for each production and quality control facility were prepared for each of the plant sizes along with a diagramatic flow sheet (Figures 2 and 3). Manning tables, capital, and operating cost budgets were prepared for each size production facility.

Cost data for plant, equipment, processing and quality control indicated that in Pakistan tea could be fortified with Vitamin A at 250 IU/g for approximately $0.007 per pound, using the data for a 500,000 pound concentrate (20 X) per year plant and at about $0.006 per pound for the larger size concentrate plants. On a per

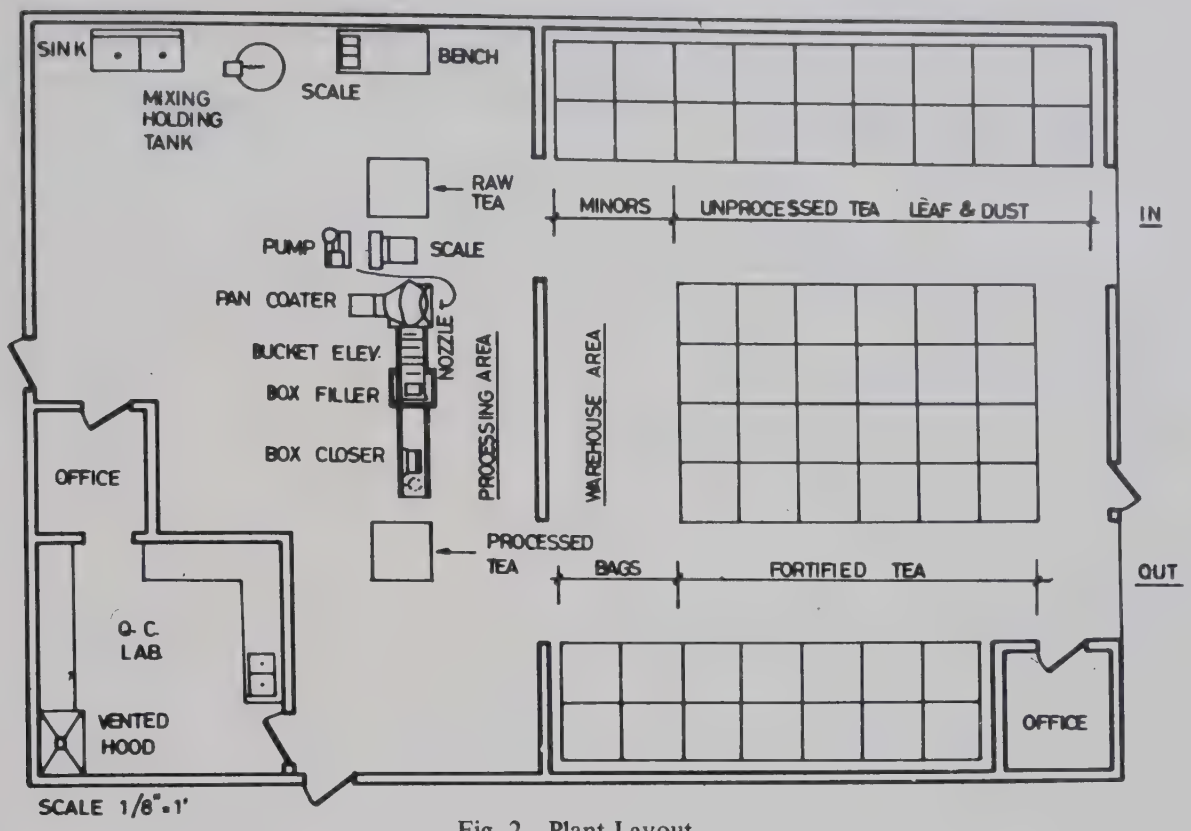


Fig. 2.–Plant Layout.

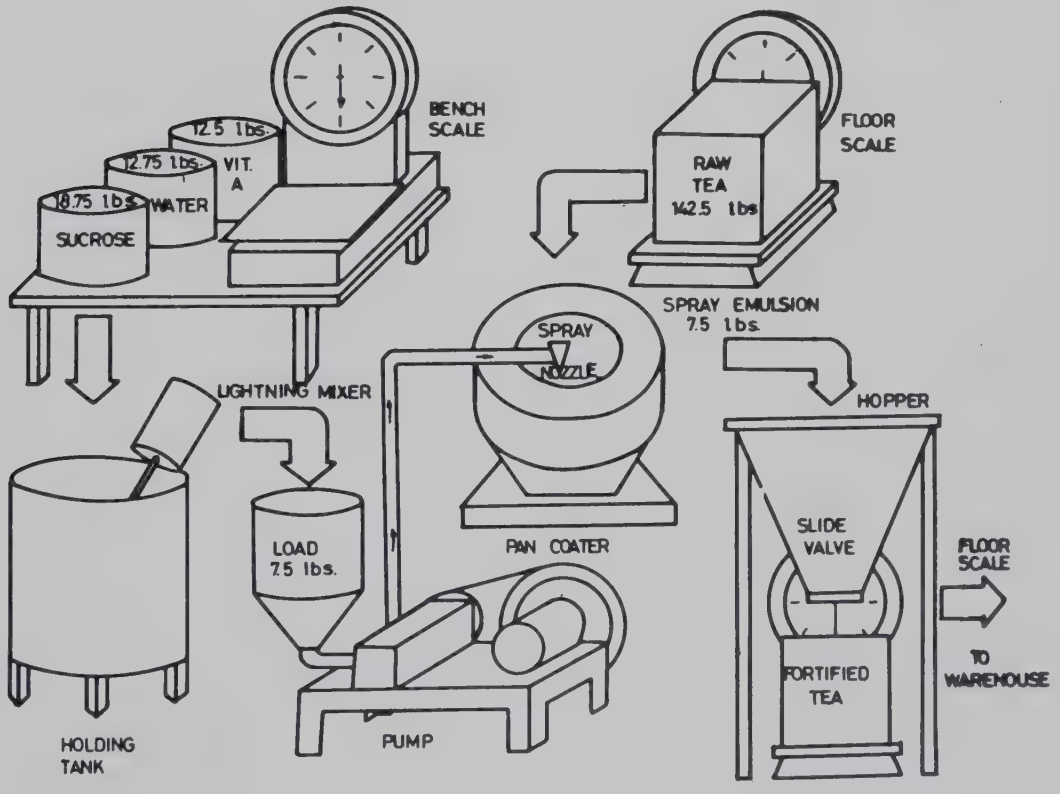


Fig. 3.–Diagramatic flow sheet.

TABLE 6

Estimated cost of fortifying tea, with 500,000 pounds per year concentrate plant

ITEM	Cost per pound ($)
Total ingredients.	0.698
Quality control costs.	0.011
Labor costs.	0.009
Maintenance (@ 15 % equip. cost/yr.).	0.006
Depreciation (bldg. 20 yr., equip. 10 yr.).	0.012
Utilities.	0.001
Total manufacturing costs.	0.737
Incremental cost.	
(Replacing tea with concentrate @ $0.60/lb.).	0.137
Costs of fortifying @ 250 IU/g.	0.0068

cup basis (3 g) this would be $0.000045— a very small cost. (Table 6).

A suggested strategy for implementation of the project was developed and called for a three year time frame during which four plants would be established, one each in the major metropolitan areas to serve the tea trade throughout Pakistan. They would be private businesses, two of which would serve the packaged tea trade and the other the loose tea trade. The responsibilities of each of the participants —the Government of Pakistan, U. S. A. I. D. and private corporations— were detailed as to legal, financing, provision of consulting and other services, and other requirements. The three year cost of the project was estimated to be approximately $530,000 of which over $200,000 would be Rupees. In order to evaluate the effectiveness of this program and assure the continuation of the program, additional studies were suggested. They were: 1) a study to determine the retention of potency of fortified tea stored under ambient conditions in Pakistan, 2) a study to determine the levels of amelioration of Vitamin A deficiency symptoms in sample population consuming only fortified tea, and 3) a study to determine the feasibility of the production of Vitamin A in Pakistan. In addition, two preliminary or pre-implementation studies were suggested to provide data which would aid in the establishment of fortification levels, both of which were directed at the target market. One of the studies dealt with the tea purchase-consumption habits of the population and the other with an update of the deficiency levels of Vitamin A among children and pregnant and/or lactating womem.

In summary, the technology developed for the fortification of tea with Vitamin A which was developed was one which accomplishes the task at a low cost. In addition to the low cost, it is a simple technology which can easily be transferred as well as requiring equipment for production of the concentrate that is easily constructed by local fabricating firms. Finally, the use of concentrate (heavily fortified tea materials) can easily be blended into final product with the existing equipment of local tea blending plants.

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Worldwide applications for textured vegetable protein as meat replacements

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ABSTRACT

Textured soy protein products containing 50-60 % protein have been successfully incorporated as lean meat replacements in a variety of meat products. Formulations have been developed with meat processors in Latin America, Western Europe, and the United States.

Formulations for lean meat replacement of 15-30 % have been developed for two principal types of meat products of Latin America and Western Europe; namely, embutidos and fiambres. In some instances, up to 100 % lean meat replacements have been implemented. Textured soy protein applications in the United States have focused primarily on their use in meat patties. Replacement levels for up to 45 % lean meat have been realized through the use of textured vegetable protein containing spun soy fibers. Independent laboratory test results on samples of meat and textured soy protein blends containing 45 % hydrated soy protein have demonstrated a PER favorable to a casein reference standard.

Our experience in meat processing plants in over 12 countries has shown that generally no processing changes are required when textured soy protein products are utilized in meat formulations.

INTRODUCTION

In recent years there has been considerable interest in the development of protein foods from other than animal origin. Some of these alternate sources of non-meat protein are more conventional than others and have been quickly accepted. Over the past four years we have observed the owerwhelming acceptance of textured soy protein as a rich source of high quality protein that is both savory and economical. The affirmative response to these products has also been due, in part, to their compatibility when blended with meat products.

Soybean processing begins in the fields. From millions of acres world-wide, oil-rich, high protein soybeans are harvested. These beans, yellow or green in color, round and fat, and somewhere between the size of a pea and a grape, go into modern processing centers for crushing into soybean oil and meal.

Soybeans may be extracted to produce soybean oil and oil-free flakes which are the basis of soy flour (50 % protein), soy protein concentrate (70 % protein), and isolated soy protein (95 % protein). These protein sources are then used to manufacture textured soy protein and spun soy protein. The two main forms of textured soy proteins are analogs and dehydrated granules. Analogs are simulations (analogous to) of ham, beef, chicken, and other typical meats. They are produced by combining soy isolate fiber, other proteins, flavors, colors, binders, and cooking the mixture. Final products have flavor, nutrition, texture, and appearance closely resembling meat. However, analogs must be held and transported frozen, which limits their worldwide applications.

The textured vegetable protein granules, on the other hand, are produced by the thermoplastic extrusion of soy flour. This unique cooking-extrusion process imparts a meat-like appearance and texture to the final product. Under carefully controlled conditions, the thermoplastic protein mass is extruded under pressure through special dies which dictate the shape and size of the texturized product. A more sophisticated product more closely analogous to ground meat can be produced by the extrusion of a premix of soy isolate fiber and soy concentrate.

This paper which discusses some of our textured soy protein applications programs in the United States, Western Europe, and Latin America will focus principally on the extruded granules intended as extenders for animal protein.

RATIONALE FOR USE

Textured soy protein provides an excellent nutritional source, imparts functional and textural qualities, and offers economy. The following reasons indicate why soy protein has been, and will continue to be an attractive food ingredient.

Texture soy protein is well documented in the literature as having an excellent nutritive value including a minimum of 50 % protein. It contains all of the essential amino acids as well as good levels of vitamins and minerals and has a PER (protein efficiency ratio) value of at least 80 % that of casein. The ingredient composition and extrusion process are controlled to produce a uniform product. Vitamin and mineral fortification can be introduced accordingly as dictated by regulations or specific regional needs.

Textured soy protein is compatible with the texture of ground meat. When blended with meat, it maintains flavor and texture yet minimizes shrinkage by the retention of the juices in the ground meat mixture. The granules can be colored or flavored prior to extrusion or during hydration by the addition of coloring and/or seasoning to the liquid used for hydration.

Soy proteins are easy and convenient to use. When hydrated with liquid the textured granules acquire a chewy, meat-like texture. The granules can then be easily blended or emulsified with meat using conventional methods. Hydrated textured vegetable protein produced from extruded soy flour can replace up to 30 % meat in most products with minimum textural differences. Hydrated textured vegetable protein produced from the extruded premix of soy fiber and soy concentrate can replace up to 45 % meat in many products. These resultant meat/soy mixtures have a PER equivalent to that of casein.

Low-cost textured soy protein can be blended with meat protein to provide a finished product with comparable nutrition and palatability as meat, but at significantly reduced cost enabling it to be within the economic reach of a greater number of consumers.

In dry form, textured soy proteins offer several logistic advantages over meat. Because they exhibit long-term shelf stability without refrigeration, they can be conveniently packaged, transported, and stored with minimum cost.

The use of textured vegetable proteins has gained increasing acceptance as nutritious, savory (tasteful), and economical protein foods. The challenge to offset the threat of an impending protein crunch in the 1980's, together with industrial and governmental commitments to health and nutrition have added momentum to the effort to develop supplements, extenders, and alternates from non-meat sources.

UNITED STATES

During the last three years in the United States, textured vegetable proteins have made dramatic advances in the marketing and consumer acceptance of meat/textured vegetable protein blends. The first significant breakthrough in the United States for blending textured vegetable protein with meat was realized in February, 1971, when the USDA Food and Nutrition Service authorized the use of textured vegetable protein fortified with minerals and vitamins as a 30 % meat alternate in the meat requirement for the Type A School Lunch.

According to USDA's Food and Nutrition Service Notice 219 (USDA, 1971), the ratio of hydrated vegetable protein product to uncooked meat, poultry, or fish in combination must not exceed 30 parts to 70 parts, respectively, on the basis of weight (i. e., 30 % of the total uncooked meat may be replaced with hydrated textured protein products).

The USDA established certain requirements for protein, fat, minerals, and vitamins. The PER of the textured vegetable protein must not be less than 1.8 on the basis of a PER of 2.5 for casein, or equivalent to 72 % of casein PER. The PER of the meat/textured vegetable protein mixture must not be less than 2.5.

Confidence in the nutrition and quality of soy proteins was further demonstrated last year when two additional breakthroughs of major significance were realized: 1) the commercialization of a textured protein product and fresh meat mixture merchandized through supermarket meat counters and, 2) the marketing of consumer packages of textured vegetable protein designed to be hydrated and blended with meat in the home. Both set the precedent for voluntary consumer acceptance for meat/meat alternate blends. For the first time, consumers were offered an economical product comparable in nutrition and flavor to ground beef. Continued consumer acceptance of these products has helped created a favorable marketing climate for soy proteins in the United States.

From an industry standpoint, application of textured vegetable protein in the U. S. has been heavily concentrated on the meat patty industry. This represents a sizable market that has proven readily acceptable for penetration with textured soy protein. Replacement of ground beef with textured soy protein is simple and straightforward. Hydrate the textured soy protein (2 parts water to 1 part protein by weight). Add the hydrated protein, beef, and beef trimmings to a chopper or grinder and chop or grind until blended. Form into meat patties. Production of meat patties replacing an equal portion of meat with 30 % hydrated textured soy flour has become commonplace. Quality patties extended 45 % can be produced with the textured soy protein extruded from a premix of soy isolate fiber and concentrate. These patties require the addition of a spice blend flavoring system to support the meaty flavor.

Textured soy proteins offer similar nutritional, functional, and economical

advantages, in a variety of dishes prepared in schools, in the home, and in institutional and convenience foodservice operations: meat loaf, meatballs, chili con carne, barbecues, spaghetti sauce, and pizza toppings to name a few.

Increased application work has been directed toward production of quality frankfurters using 30 % textured soy flour or 30 % textured fiber/concentrate premix. A typical formulation is shown in Table I.

TABLE 1

Frankfurter with 30 % textured soy protein*

Ingredients	Percent (%)
Textured Soy Protein (Hydrated).	30.0
Beef Shank.	20.7
Water.	14.0
50 % Pork Trim.	13.5
80 % Pork Trim.	9.6
Beef Flanks.	6.3
Seasoning and Curing Aids.	5.9
	100.0

Procedure:
1. Grind meats and extender together.
2. Add salt and mix.
3. Hold to allow adequate myosin extraction (for binding capability).
4. After extraction, add seasonings and water.
5. Emulsify, stuff, and cook.

*** In U. S. these products cannot be called frankfurters, but must carry a "fanciful name".**

The finished frankfurters were subjected to both cooking and taste panel testing. The extended frankfurters were judged similar to the all-meat control in flavor and overall eating acceptability. When subjected to a variety of cooking processes, the extended frankfurters responded similarly to the all-meat control.

During this past year, we have also seen the commercialization of bologna and pepperoni extended with textured vegetable protein. A typical bologna formula listed in Table II produces a product with good texture, appearance, and flavor.

WESTERN EUROPE AND LATIN AMERICA

Our applications technology activities in Western Europe and Latin America have been aimed at demonstrating the functional, nutritional, and economic opportunities realized through the use of textured vegetable proteins. Two main categories of provision meat products in Western Europe and Latin America are embutidos (dry types) and fiambres (cooked meats). As a result of our work with meat processors in Western Europe and Latin America, textured vegetable protein demonstrates important application results. From a manufacturing standpoint, these protein products displayed functionality through incorporation into an emulsion or inclusion to represent particulate pieces of meat. When incorporated into the emulsions, they have the capability of extending the system, yet develop or maintain the desirable consistency. Textured vegetable protein granules can also be preselected on the basis of particle size and incorporated without reduction in size to

TABLE 2

Bologna with 30 % textured soy protein*

Ingredients	Percent (%)
Textured Soy Protein (Hydrated).	30.0
80 % Pork Trim.	23.0
Beef Shanks.	17.2
Water.	17.1
Beef Flanks.	6.7
Seasoning and Curing Aids.	6.0
	100.0

* In U.S. these products cannot be called bologna, but must carry a "fanciful name".

serve as distinct meat alternate chunks. Because textured vegetable proteins are stable under retort and smokehouse conditions, they are of considerable value to canners and meat processors.

Textured soy proteins were demonstrated to be successful as meat replacements in most of the traditional meats found in these two heavily populated areas of the world. Hamburgers, although far more complex in composition than their American counterparts, were found to be of increasing importance as a meat protein food.

Tables III, IV, V, VI, VII and VIII contain typical formulations of these traditional processed meat products which were produced with significant levels of textured soy protein as meat replacements.

TABLE 3

"Frankfurters" with 30 % and 50 % meat replacement

	Replacement level	
Ingredients	30 Percent (%)	50 Percent (%)
Beef.	24.0	17.0
Fatty Pork.	13.0	9.5
Textured Soy Protein (Hydrated). .	16.0	26.5
Pork Fat.	6.5	6.5
Ice/Plasma.	30.0	30.0
Flour.	8.7	8.7
Salt, Curing Agents, Sugar, Phosphates, Spices.	1.8	1.8
	100.0	100.0

GOVERNMENT REGULATORY ENVIRONMENT

The United States regulations have been generally favorable for the timely inclusion of textured vegetable proteins in meat mixtures. U. S. regulations governing food ingredients and labeling reflect continued government regulatory activity for the protection of the consumer.

TABLE 4

"Salchichas" with 20 % meat replacement

Ingredients	Percent (%)
Beef.	43.0
Textured Soy Protein (Hydrated).	10.7
Flour (Corn and Wheat).	8.6
Pork Fat.	6.5
Ice.	30.1
Phosphates, Sugar, Salt, Curing Agents, Spices.	1.1
	100.0

TABLE 5

"Mortadella" with 30 % meat replacement

Ingredients	Percent (%)
Beef.	23.0
Pork.	10.5
Textured Soy Protein (Hydrated).	15.0
Fat.	26.5
Potato (Dehydrated).	10.0
Water/Ice.	14.0
Spices.	1.0
	100.0

TABLE 6

"Frankfurter" containing no lean meat

Ingredients	Percent (%)
Fat Slabs.	28.0
Textured Soy Protein (Hydrated).	20.0
Skin Emulsion.	6.3
Soy Isolate.	6.3
Potato (Dehydrated).	3.8
Ice/Plasma.	35.6
Spice/Color.	–
	100.0

TABLE 7

"Chorizo" with 30 % meat replacement

Ingredients	Percent (%)
Beef, lean.	40.6
Pork, fatty.	39.0
Textured Soy Protein (Hydrated).	17.4
Seasoning, Curing Agents and Color.	3.0
	100.0

TABLE 8

"Hamburger" formulation with 28 % textured soy protein

Ingredients	Percent (%)
Meat.	50.0
Textured Soy Protein (Hydrated).	28.0
Fat.	4.0
Bread Crumbs.	2.0
Water.	9.0
Vegetables.	5.0
Seasonings.	2.0
	100.0

Procedure:
1. Add vegetables, seasonings and bread crumbs to chopper.
2. Add coarse ground or flaked meat.
3. Add hydrated textured soy protein (extender).
4. Add water.
5. Add fat, and chop sufficiently to mix.
6. Grind mixture through 8 mm plate.
7. Form patties.

The regulatory climate in many countries has not reflected the tremendous progress being made in protein research, processing and texturizing technologies, nutrition, and applications. In many countries, specific regulations governing the use of textured protein ingredients have not been developed or implemented.

Latin American countries generally have not formalized any regulatory position or philosophy on the use of textured vegetable protein. In most of these countries, products containing textured vegetable protein need only be authorized by the government and/or comply to existing food regulations.

In the Western European countries, food and labeling regulations regarding textured vegetable protein are, for the most part, in the formative stages. However, the existing regulations generally restrict the blending of textured vegetable protein with meat.

CULTURAL AND ECONOMIC FACTORS

Much of the worldwide acceptance of textured vegetable protein is credited to its ease of addition and its general compatibility with most traditional foods as well as its adaptability to socio-economic environments. We can be optimistic about the worldwide acceptance of textured soy extended meat products for the following reasons.

1. The voluntary acceptance of textured soy protein by an affluent society has been demonstrated in the United States by the purchase of prepackaged extended ground meat and packaged dehydrated extenders. Textured soy protein has been positioned as high quality meat alternate protein available at a relatively low price. Efforts to establish and reinforce textured soy protein as a food acceptable to a cross section of an affluent society, one that has traditionally been rated as having the highest per capita consumers of meat, have been successful. We believe that textured soy protein blended with meat can become a classless product, one that will be accepted by most all segments of a society alike.

2. In many portions of the world, the economic environment is changing. Economic development accompanied by rising income levels has imposed increased demands on the world's supply of animal protein. It is well known that most of the world derives the majority of its protein from cereal sources. However, it is also known that with affluence emerges stronger demands for protein in the form of meat. A realistic compromise appears reasonable for the acceptance of textured soy/meat blends by those of emerging affluence who are traditionally consumers of a large percentage of cereal based foods.

3. Textured soy proteins blend well with meat and are therefore compatible with most traditional ethnic meat products prepared from ground meat or meat emulsions.

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The potential value of algae as a food supplement

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ABSTRACT

One of the factors that discredits the algae as a potential food supplement is their intense colouration. A number of processes that might be useful for decolourisation are available and the possibilities offered by solvent extraction have been considered in detail. Ethanol is considered to be the most appropriate organic solvent for the removal of chlorophylls and xanthophylls. Powders, 'off-white' in colour and with a neutral odour, have been obtained by processing a number of algae, and it is proposed that such materials would be complementary to cereal flours. Some aspects of the nutritional quality of the refined products have been assessed by analytical means, and found to be comparable with the 'raw' material. Nutrients other than protein have been monitored, e.g. fatty acids and vitamins, and it is suggested that the presence of these nutrients could make the algae a valuable dietary supplement irrespective of any considerations concerning protein.

INTRODUCTION

In recent years, much has been written about the present inadequacy of food supplies in the world, and there is widespread concern that the situation will deteriorate still further. This paucity of available supplies is obviously not uniform, but in certain areas, malnutrition is a critical dietary problem. In some instances, this problem is due to a lack of "food", but in others, it is due to a nutritional deficiency of the staple diet. Such a deficiency frequently involves protein, although a low intake of vitamins or essential fatty acids can be equally serious. However, the most urgent need appears to be to increase the production of edible plant protein, particularly in areas where it is most needed.

It is against this background that the algae merit serious consideration as a material with which to supplement an otherwise deficient diet. Thus, not only does the productivity of algal cultures compare very favourably with conventional agriculture, (Vincent, 1969), but also, the algae could well be produced on marginal land areas quite unsuitable for farming, (Robinson, 1973). The nature of the harvested material with its intense colouration and rigid, cellulose cell walls has lead to the conclusion that the logical outlet for algae is as an animal feed. Undoubtedly this avenue of exploitation will be employed, but the possibility of using algae as a direct means of raising the nutritional quality of a diet is an interesting one.

Consequently it is the aim of this paper to assess the potential nutritional value of the algae as a food supplement, and consider how the raw material might be processed with a view to optimising its acceptability.

ALGAE AS A SOURCE OF PROTEIN

It is clear from the data presented in Table 1 that the chemical composition of cells from any given species varies considerably with conditions of growth. The range of variation reported by Milner (1953) is, to some extent, extreme, but it does indicate that the protein content of any material produced commercially will depend on the environmental conditions of the culture.

For comparison, the data for protein content has been presented as 'crude' protein (Kjeldahl x 6.25), but it is important to note that between 1 - 2 % of the total nitrogen recorded is in the form of non-protein nitrogen. It is for this reason that the possibility has been examined of employing dye-binding techniques to monitor the protein content of unicellular organisms. The advantages of this procedure has been reviewed elsewhere (Lakin, 1973), but its application for single-cell organisms has been little considered. Nevertheless, as a method of monitoring production, dye-binding could offer certain advantages over normal control measures, and its suitability for this purpose is currently being investigated.

However, perhaps even more important than the total protein value is the assessment of quality, and the amino-acid spectra for a number of algae are shown in Table 2. It is clear from this table that, in general, the algae show a deficiency of sulphur amino-acids. In practice these low levels of methionine and cysteine/cystine may not present a problem, and Ribbons (1968) showed clearly that *Chlorella* was an adequate source of protein for rats. Thus, algal protein can provide all the essential amino-acid in a diet, and could certainly be used as a supplement in human food.

TABLE 1

Protein and lipid content of certain algae

Algae	Protein (% dry wt.)	Lipid (% dry wt.)
Chlorella vulgaris.	50.6	8.9
Scenedesmus caudatus.	52	7.2
Spirogyra nitida.	46.1	–
Spirulina platensis.	64	11
Chlorella pyrenoidosa.*	88.2	5.2
	7.3	83.2
Spirulina platensis[+]	64.2	7.3

Data from: * Milner (1953);[+] Clement (1971).

ALGAE AS A SOURCE OF OTHER NUTRIENTS

The data in Table 1 also indicate that the algae can contain an appreciable amount of lipid material, but, as with protein, the quantity present in any particular sample depends on the conditions of growth. The fatty acid profile is, of course, of the utmost signficance in the present context, and it is of interest that 20 % of the lipids extracted from *Spirulina platensis* are in the form of linoleic and

TABLE 2

Amino acid composition of certain species of algae (g/100 g protein)

Amino acid (essential)	FAO (ref. standard)	*Chlorella vulgaris*	*Spirogyra** *nitida* (a)	*Spirogyra** *nitida* (b)	*Spirulina*† *maxima*
Isoleucine.	4.2	3.2	4.3	3.9	6.0
Leucine.	4.8	4.6	7.0	6.7	4.8
Lysine.	4.2	4.5	3.2	3.1	4.2
Phenylalanine.	2.8	2.6	3.5	3.2	5.0
Tyrosine.	2.8	2.4	–	–	4.0
Cysteine/cystine.	4.2	0.5	–	–	0.4
Methionine.	2.2	0.7	0.5	0.4	1.4
Threonine.	2.8	2.6	4.2	3.9	4.6
Tryptophan.	1.4	1.0	–	–	1.4
Valine.	4.2	4.7	5.0	6.3	6.5

† Data from Clement et al. (1967).
* (a) normal sample; (b) decolourised with ethanol.

linolenic acid (Hudson, 1974). These fatty acids are regarded as essential in al diet, and if their presence is confirmed in other algae of potential commercial significance, then the supplementation of a diet, otherwise deficient in essential fatty acids, could be beneficial apart from any considerations concerning protein.

It may also be significant that that unicellular algae could provide a source of vitamins in a diet. Thus, not only are the B vitamins well represented, but also the the nutritionally valuable β-carotene and tocopherol (Kihlberg, 1972). In one species examined, *Spirulina platensis*, vitamin B 12 is also present, and in sufficient quantity to imply consideration of this material simply to supply a dietary requirement for cobalamin; this vitamin has also been reported in *Chlorella pyrenoidosa* (Fisher and Burlew, 1953).

ACCEPTABILITY OF ALGAE

It is clear that, if the algae are to find favour as a food supplement, then the problems of introducing the material into a diet are of primary importance. In this context, 'acceptability' can be said to encompass two components, namely the nutritional value of the material and consumer reaction.

Mention has already been made of the potential dietary value of algae as assessed by analytical techniques, and, on the whole, feeding trials support these results. Thus, Lee *et al* (1967) demonstrated that *Chlorella* can replace one-third of the protein of egg and up to two-thirds of the protein of fish without any impairment in the nitrogen retention of human adults. Reports of this type clearly indicate that while algae may not represent an 'ideal' source of protein, it could definitely prove attractive as a supplement in a diet dominated by carbohydrates.

It is of interest, however, that one of the reasons for the mediocre performance of algal protein is that it is contained in the chemically inert cell-wall, and it has been suggested that this situation could be improved by suitable processing (Gordon, 1971). If this treatment resulted also in a decrease in colouration, then the

possible problem of consumer rejection could be overcome. To this end, a series of decolourising procedures have been examined; these treatments include extraction by solvents, namely ethanol, ethanol/water (80 : 20), acetone and acetone/ethanol (30 : 70), bleaching and electrolysis. The aim of these procedures was to produce a dry, protein-rich powder with an 'off-white' colour and neutral odour; maize flour was proposed as a practical standard.

Each procedure was examined in turn against the criterion of its suitability for scale-up as a commercial operation, and in relation to its effect on the nutritional value and colouration of the end-product. The employment of these guidelines eliminated the electrolytic technique on the grounds of the projected cost of a full-scale development, while the proposed colour standard has not been achieved by any of the bleaching agents so far employed.

The most suitable procedure, in terms of decolourisation, found to date has involved the removal of pigmented materials with ethanol. However, the most satisfactory product was obtained by treating freshly harvested material, and hence the ethanol became diluted in the process; a point of some importance in relation to the suitability of the procedure for full-scale development. The reason for the apparent resistance of the dry material to treatment has not been elucidated, but the establishment of a relationship between the degree of dehydration of a sample and its ability to retain pigments is, for any given species, a practical essential. However, assuming that a satisfactory procedure can be established, then there is little doubt that the material could prove complementary to conventional carbohydrate-rich foods. One essential feature of the product must be its nutritional value, and the data presented in Table 2 indicates that decolourised algae could provide a useful source of amino-acids. If the presence of certain essential fatty-acids and vitamins proves equally encouraging, then the exploitation of algae as a food supplement would be worth serious consideration.

CONCLUSION

Although it is generally concluded that the algae will initially be exploited as an animal feed, the possibility of direct human utilisation should not be ruled out. Thus, the emergence of a totally satisfactory decolourising technique would enable the algae to be used freely as a food supplement; not only as a source of protein, but possibly to provide a dietary requirement in terms of vitamins and fatty-acids as well.

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The effect of the method of extraction on the amino-acid composition of rapeseed protein isolates

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ABSTRACT

Use of rape seed protein in human nutrition, made possible its isolation from rape seed meal by three step extraction: water – 0.04N NaOH – water, and coagulation by pH 3.9. Isolates of rape protein have a high level of cystine and methionine (4.9g/16gN), treonine (4,2), leucine (7,2) and lysine (5,8). The main limiting amino acids were: isoleucine (3,8g/16gN) and partly valine (4,8) as well as aromatic amino acids (6,8). Glucosinolates were not present.

INTRODUCTION

The increase of use of plant proteins as food additives and development of techniques employed for production of vegetable protein concentrates and isolates create new perspectives for utilization of rape-seed protein in human nutrition too.

Direct utilization of the rape-seed protein as a human food in the form of fat-free rape seed flour is impossible because of the presence of relatively high content of cellulose and glucosinolates (Rutkowski 1972). While it is possible to reduce the level of cellulose in the seed substance by sifting or winnonwing away the husks in the air stream (de Man and Stanley, 1974), yet the removal of glucosinolates enconters more serious difficulties. However the action of water solutions used by production of concentrates and isolates makes possible to remove the glucosinolates precursors of the goitrogenic compounds 5-vinyl-1,3-oxazolidin-2-thione, and isothiocyjanates having the pugent smell reacting irritatingly on the mucous membrane.

The most widely used soya-bean products, utilized as food additives are protein concentrates. Unfortunately, high contnet of starch and cellulose present, even in dehulled rapeseed is the reason for rather low protein concentration

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(50-60 %) in the rape seed protein concentrates (Tape et al., 1970), while the obtainment of soya protein concentrates containing about 70 % protein does not encounter any serious difficulties (Martinez 1968, Meyer 1968). Nevertheless it is important to emphasize that Kozlowska et al. (1972) succeeded in removal 5-vinyl-1,2-oxazolidin-2-thione and in reducing the level of isothiocyianates to about 0.5 mg/g seed mass by 3-5 fold water diffuse extraction.

Better results were obtained while getting rape seed protein isolates by the method of alkaline extraction (Rutkowski and Korolczuk 1974) and acidic coagulation of protein. in this case it was possible to remove insoluble cellulose and glucosinolates, which move into solution and remain there after coagulation.

A part from pure technological affairs,working out the method of obtaining of edible rape seed protein isolates, it is necessary to establish how technological process influences the protein quality of the obtained isolates.

MATERIAL AND METHODS

Commercial rape seed winter variety (*Brassica napus*) has been used in our experiments. It contained 96 % dry mass, 45 % fat, 22 % protein (Nx6.25). The fat was extracted from the ground seed by cold (20° C) petrol ether (b. p. 40-60° C). In order to avoid protein denaturation postextracted rape seed meal was dried at room temperature overnight and the drying was completed in a vacuum drier under the pressure of 1 mm Hg at a temperature of 40° C for 4 hours. The rape seed meal obtained in this way contained 0.2 % fat, 40 % protein, 16.7 % cellulose.

The process of protein isolation from rapeseed meal was based on the previously established (Rutkowski and Korolczuk, 1974) scheme (fig. 1) as three-stage extraction process,where as solvents were used water solutions of hydrochloric acid or sodium hydroxide of a concentration up to 0.1 N. Extracted protein was coagulated at pH 3.9.

Protein concentration and the balance of nitrogen was determined by Kjeldahl method, pH of extraction and coagulation by means of pH-meter (Radiometer). Soluble rape seed proteins were fractionated by gel filtration on Sephadex G-150 using the column 3.2 x 50 cm, buffer 0.1 M di-sodium tetraborate, by means of Re-Cy-Chrom (LKB) chromatograph. Amino acid composition was determined after 20 h. hydrolysis in 6 N hydrochloric acid at 110° C by Spackman et al. (1958) by means of automatic amino acid analyser (Technicon). Sulphur amino acid were determined after initial oxidation to cysteic acid and methionine sulphone by Schram et al. (1954).

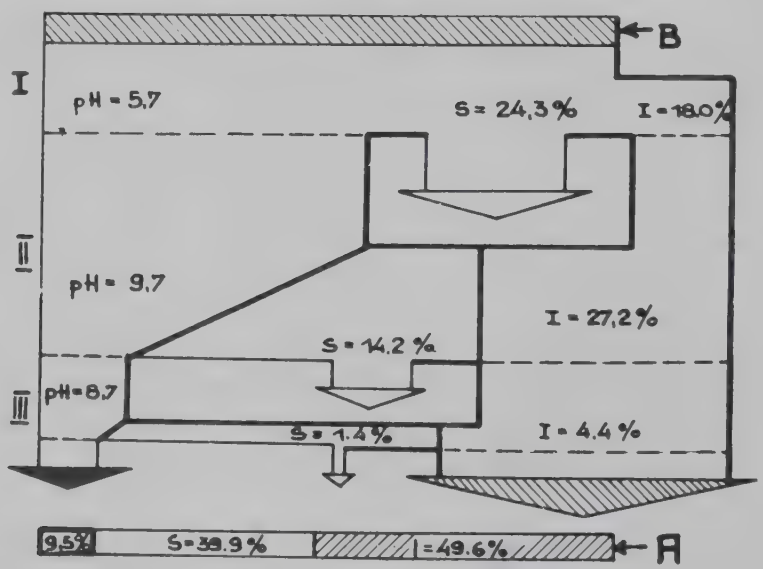


Fig. 1.–Scheme of three-stage extraction of rapeseed proteins.
The yeld of proteins examined in % of N.
A = Balance of extracted proteins, B = Protein in starting material
■ = Insoluble proteins, □ = Soluble (S) proteins, noncoagulated by pH 3.9, ▨ = Protein isolate (I) coagulated by pH 3.9.

RESULTS AND DISCUSSION

Thee-stage extraction (pH 5.7 - 9.7 - 8.7) comparing with the other methods enables relative high yield of isolation (about 50 % total nitrogen). Only about 10 % of meal nitrogen was not soluble, and as far as 40 % total nitrogen constituted noncoagulating nitrogen substances. The yield of protein isolates and the level of noncoagulating nitrogen compounds are similar to the values obtained by Pokorny and Vodicka (1964) and Sosulski and Bakal (1969).

The examination of the soluble nitrogen compounds by Sephadex gel filtration (fig. 2) showed, that in the solution after the first step of water extraction (pH 5.7) there were two protein fractions (A and B) of the molecular weight on the level of 100.000, and low molecular compounds (C) in the range of molecular weight about 1.000.

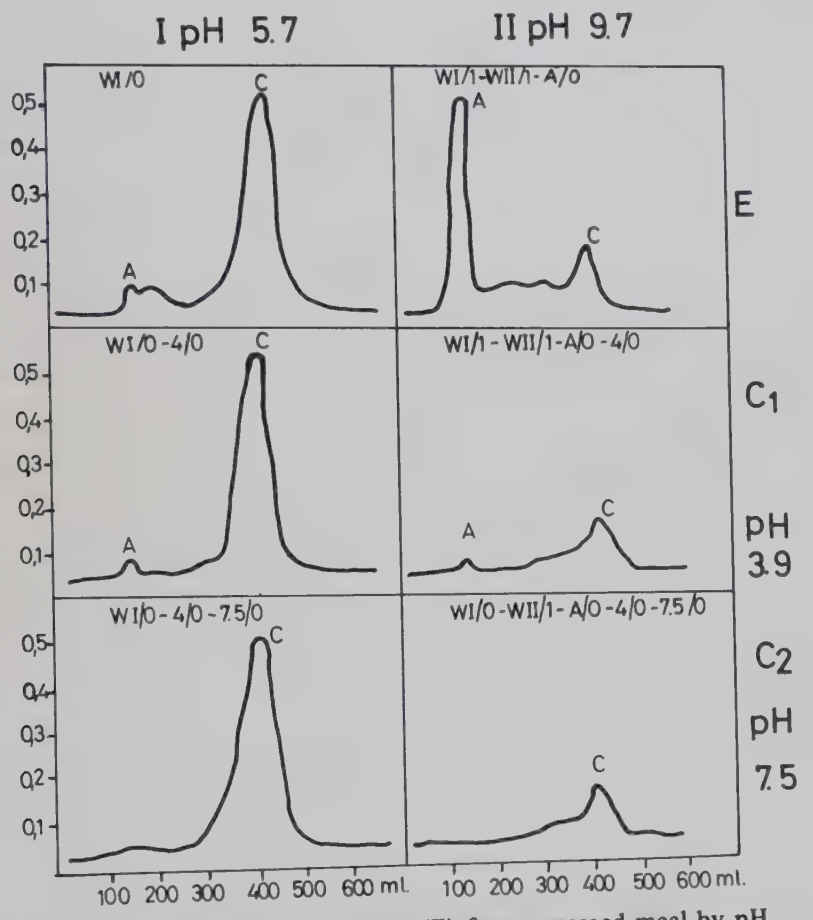


Fig. 2.–Protein fractions in extract (E) from rapeseed meal by pH 5.7 (I) and pH 9.7 (II), and remained in supernatant after coagulation by pH 3.9 (C_1) and pH 7.5(C_2).

After the second stage of alkali extraction (pH 9.7) the main component in the solution was protein fraction (A). After coagulation of the protein at pH 3.9 both in water and alkali extracts only traces of high molecular compounds (A) were found, and the initial level of low molecular substances (C) remained unchanged. The introduction of additional coagulation at pH 7.5 resulted in all proteins being coagulated and in the supernatant only the low molecular components (C) remained at the same initial level.

The results obtained prove the our former conclusion (Rutkowski, Korolczuk 1972) that as well as the main protein fraction coagulating at pH 3.9, there is the other fraction coagulating at pH 7-8. This fraction, however, constitutes only about

TABLE 1

Essential amino acids of rapeseed protein isolates (% of protein)

Extraction Coagulation	Rapeseed protein	Water pH 5.7 NaOH pH 9.7 Water pH 8.7 → HCl pH 3.9	Water pH 5.5 Water pH 5.7 Water pH 6.1 → HCl pH 3.9	HCl pH 1.9 → NaOH pH 3.9	HCl pH 1.9 → 7.5	NaOH pH 9.7 Water pH 9.1 of protein)	Soybean isolate Promine	Hens egg protein
Isoleucine.	2.5	3.8	4.2	3.9	2.9	3.9	4.8	6.6
Leucine.	5.6	7.2	8.2	7.5	5.2	7.5	7.6	8.8
Lysine.	4.9	5.8	6.4	5.4	6.8	5.4	6.0	4.4
Cyst. + Met.	5.4	4.9	5.0	5.1	–	5.1	2.6	5.5
Threonine.	4.0	4.2	4.9	5.3	3.7	5.3	3.7	5.1
Tryptophan.	1.6	1.6	1.6	1.6	–	1.5	1.4	1.6
Valine.	3.5	4.8	5.3	5.3	4.0	5.4	4.8	8.3
A. A. - Aromat.	8.6	6.8	7.9	8.4	3.9	–	9.3	10.0
%N/16 gN.	3.5	14.0	14.5	12.8	3.0	–	14.0	–

1 % of the total nitrogen, and does not in effect play any significant role in forming the yield of protein isolation from rape seed meal.

A comparison of the amino acid composition of the rape seed meal and protein isolates (tabl. 1) showed, that the isoltes are generally characterised by higher level of essential amino acids than the initial meal. From this a conclusion can be drawn that from the nutritional point of view the rape seed protein is characterised by more beneficial amino acid composition than noncoagulating low molecular nitrogen fraction. So that the properties of rape seed protein are different and more beneficial than those which occur while isolation of soy bean protein, which isolates are characterised by the lower essential amino acid level, and especially sulphur amino acids.

Protein isolates obtained from the rape seed meal characterised by high level of sulphur amino acids, threonine, leucine and lysine which are most often the limiting amino acids in many food. products. The content of the above mentioned amino acids was similar to those in whole hens egg. Only the isolates obtained by the direct alkali (pH 9.6) or acidic (pH 1.9) extraction indicated slightly lower content of lysine. The main limiting amino acid in the isolates obtained were isoleucine and partly valine and aromatic amino acids. There were no essential differences in amino acid composition of the protein coagulated at pH 3.8 regardless of the pH of extraction the protein from the meal.

The protein isolates obtained in the first two stages of water, alkali extractions (pH 5.7 - 9.7) were characterised by bright colour and the highest content of essential amino acids. They contained 14-15 % of nitrogen in dry mass, and the essential amino acid index was of the level of 80-90, and was similar to animal proteins. So that the process of two-stage protein extraction seems to be noteworthy as allowing to obtain the high yield of high nutrition quality protein isolates.

Distinct difference in amino acid composition indicated the isolate obtained by additional coagulation at pH 7.5. This isolate was characterised generally by the lower level of the essential amino acids and low concentration of nitrogen, only 3-5 % nitrogen in dry mass. Because it constituted only about 1 % of total meal nitrogen so it has not practical effect on the amino acid composition of rape seed protein isolates.

In all samples of rape-seed protein isolates isothiocyanates and oxazolidinethione (in amount more than 0.1 mg/kg) are not detected.

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Functional protein concentrates from plant and animal sources by low temperature solvent extraction using an IPA-Hexane solvent system

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ABSTRACT

Low temperature extractions of wet fatty materials which are high in protein content with appropriate solvents, produced highly acceptable protein concentrates with excellent functional properties. Simultaneous extractions of water, fat, and low molecular weight carbohydrates was possible using isopropyl alcohol (IPA), but at low temperatures solvent requirements were excessive because of poor fat solubility. The process for desolventization of the protein was very involved because of the affinity of the IPA to the protein. Extractions using IPA-hexane in the first stage followed by hexane in the succeeding stages to remove residual IPA and fat, produced excellent protein and purified fat. Desolventization procedures for both fat and protein involved simple evaporation or air drying at atmospheric pressure. Solvent recovery in a counter-current extraction system, involved dilution of the first stage miscella with water to enable phase separations into a hexane-fat phase and a IPA-water-carbohydrate phase, and recovery of hexane and IPA by evaporation and fractional distillation respectively. Proteins from poultry and beef by-products produced using this process had excellent binding properties for fat in comminuted meat formulations. The IPA-hexane solvent extraction process has also been used to produce low carbohydrate protein meat from soybeans.

INTRODUCTION

It is generally recognized that solvent extracted animal protein concentrates now commercially produced are non-functional, and because of poor wettability and solubility, blending with other ingredients is a problem (Barse, 1968; Roels, 1969). It has been shown that low temperatures solvent extractions can be used to produce functional protein concentrates from fish or other animal sources (Toledo, 1973; Dubrow, *et al.*, 1973). The problem with low temperature extractions using isopropanol (IPA) is the low solubility of fat in this solvent and thus excessive solvent is required for successful fat extractions (Toledo, 1973).

IPA is very difficult to remove from the protein concentrate. Dubrow *et al.*, (1973), working with fish proteins, reported that drying the extracted protein concentrate at 45-55 C for 30 minutes under a vacuum, resulted in a residual of 1 % IPA by weight. They made a comment that to reduce the IPA residual to a maximum of 250 ppm as required by the United States Food and Drug Administration (Federal Register, 1967), vacuum steam stripping would be necessary and this can destroy the functional properties of the low temperature exracted protein concentrates. Toledo (1973) employed vacuum drying at 40 C for 2 hours followed by moist air at 40 C for 30 minutes and final drying with dry air at 40 C for 30 minutes. This desolventization process allowed the production of functional protein concentrates and the IPA residual was at a level where samples heated inside closed jars at 50 C for 2 hours did not release detectable odor of IPA in th headspace immediately upon opening of the jars. No quantitative determination of IPA residual was performed by Toledo (1973).

In addition to desolventization problems in the production of functional protein concentrates by IPA extractions, refining of the extracted oil or fat also presented some problems. Harris, *et al.* (1949) reported that when using IPA on cottonseed, the miscella contained not only oil but carbohydrates and other non-oil extractives. When the solvent was stripped from this miscella, a large amount of the non-oil extractives remained in the oil which caused it to be dark, difficult to refine, and had a high refining loss. Harris *et al.* (1949) recommended using a liquid-liqid extraction system, using hexane, on the IPA miscella to produce good quality oil from cottonseed.

There appears to be some merit in the use of mixed solvents. In the case of oilseeds, simultaneous extraction of oil and oligosaccharides can be achieved. Oligosaccharides in beans are responsible for flatulence when the beans are ingested by humans or animals (Steggerda *et al.*, 1966). In the case of oily or fatty wet materials such as animal tissues or wet oilseeds such as fresh mature coconut meat, simultaneous extraction of water and oil can be achieved.

Extractions using two solvents have been reported by Cobb and Hyder (1972) where ethyl alcohol and hexane was used, and by Schweiger (1973) who used IPA and hexane or mixtures of polar and non-polar solvents. In both processes reported, the same solvent system was used in all stages in the extraction process. Since alcohol is still present in the solids at the last extraction stage, the desolventization procedures for both the proteins and the oil when using these solvent systems, would have to be just as involved as that for a single solvent extraction system using IPA.

In this report, a complete process for extraction, oil and protein desolventization, and solvent recovery, is described for an extraction system using IPA and hexane. The solvent requirements and the properties of the products obtained using this new process are compared with those in single solvent IPA extractions.

EXPERIMENTAL

Materials

The materials used for extraction and their fat and moisture contents are shown in Table 1. Pork back fat and beef trimmings were both obtained from the meat laboratory of the University of Georgia. Chicken meat was mechanically deboned from backs using a "Bibun" mechanical deboning machine and was obtained frozen from a local processor. Chicken preen glands were collected from the processing line of a local poultry processing plant. All materials were ground twice through a meat grinder fitted with a plate having 32 mm dia. holes, prior to extraction.

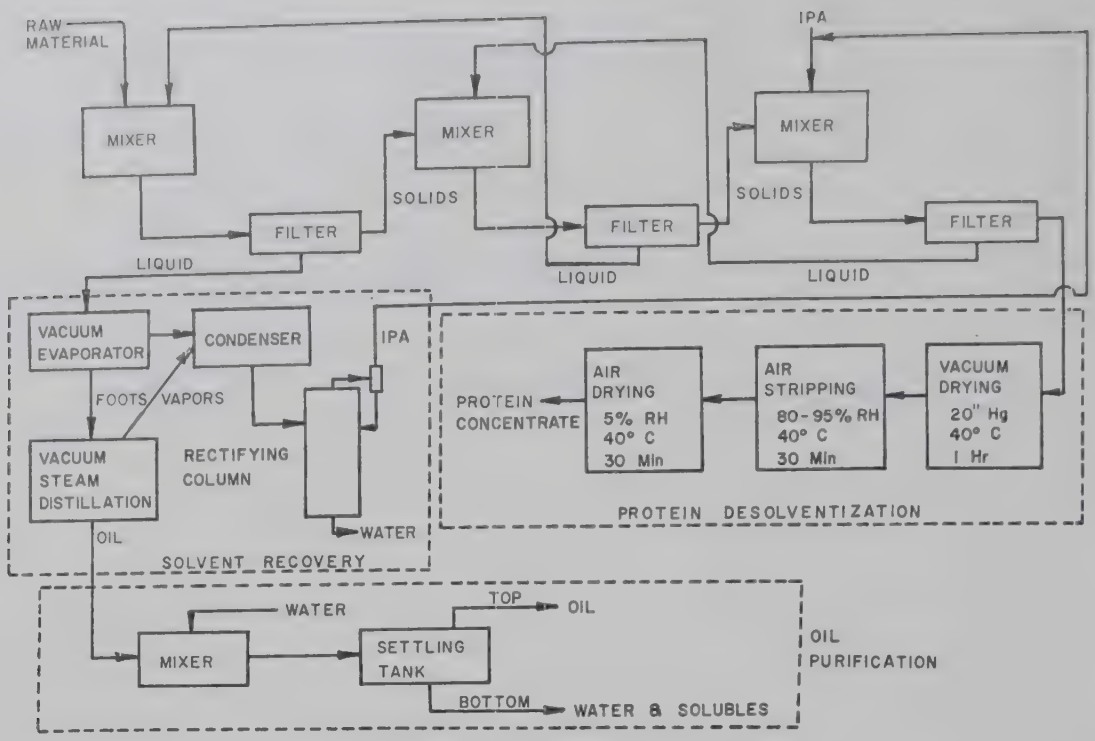


Fig. 1.–Flow diagram for protein concentrate production including solvent recovery and oil purification using isopropanol as the solvent.

Whole dry soybeans were obtained from a local producer. The beans were soaked in water at 24 C overnight, and ground through a meat grinder fitted with a plate having 32 mm dia, holes prior to extraction.

Extraction procedure for IPA extractions

Extraction and desolventization procedures were exactly the same as that described by Toledo (1973). The flow diagram for the process is shown in Figure 1. All extractions were carried out at 30 or 50 C.

Extraction procedure for IPA-Hexane extractions

The flow diagram for the IPA-Hexane extraction process in shown in Figure 2. IPA was mixed with hexane coming from the later extraction stages, prior to addition to the material being extracted in the first extraction stage. IPA is used to dehydrate the material, and hexane is utilized as the fat solvent. The major difference between this process and those previously reported, is in the use of a single solvent (hexane) in the last two stages of extraction. In these last two stages, the hexane not only extracted the residual fat but also removed the alcohol from the protein. Since the residual solvent in the protein was primarily hexane, the desolventization process for the protein was simplified.

Solvent requirements were determined from the fat and water contents of the materials extracted. The quantity of IPA required was that which allowed a maximum ratio of water/(IPA + water) of 0.3. This ratio was observed to allow sufficient dehydration of the extracted material such that separation from the miscella was simplified. Further more, hexane penetrated the solids to extract residual fat in the subsequent extraction stages. The quantity of hexane that was needed depended upon the fat content of the material extracted, the level of allowable residual fat in the protein, and the number of extraction stages utilized. For oilseeds and for most animal tissues, a 1 : 2 meats to hexane ratio was sufficient to allow a residual of less than 0.5 % fat in the protein after 3 extraction stages. For materials having very high fat contents, residual fat in the proteins less than 1 % was attained after 3 extraction stages when the ratio fat/(hexane + fat) was not allowed to exceed 0.2.

Desolventization of the protein was achieved by spreading the finely ground solids in a layer approximately 7 mm deep on a tray and exposing to dry air (5 % relative humidity) at 40 C and a velocity of 350 cm/sec for one hour. After this treatment, the odor of the solvent was undetectable when the desolventized powder was heated in a closed glass jar at 55 C for 3 hours and the headspace sniffed immediately after opening. It has also been observed that if the particle size of the solids are larger, a longer drying time is necessary to achieve the necessary desolventization.

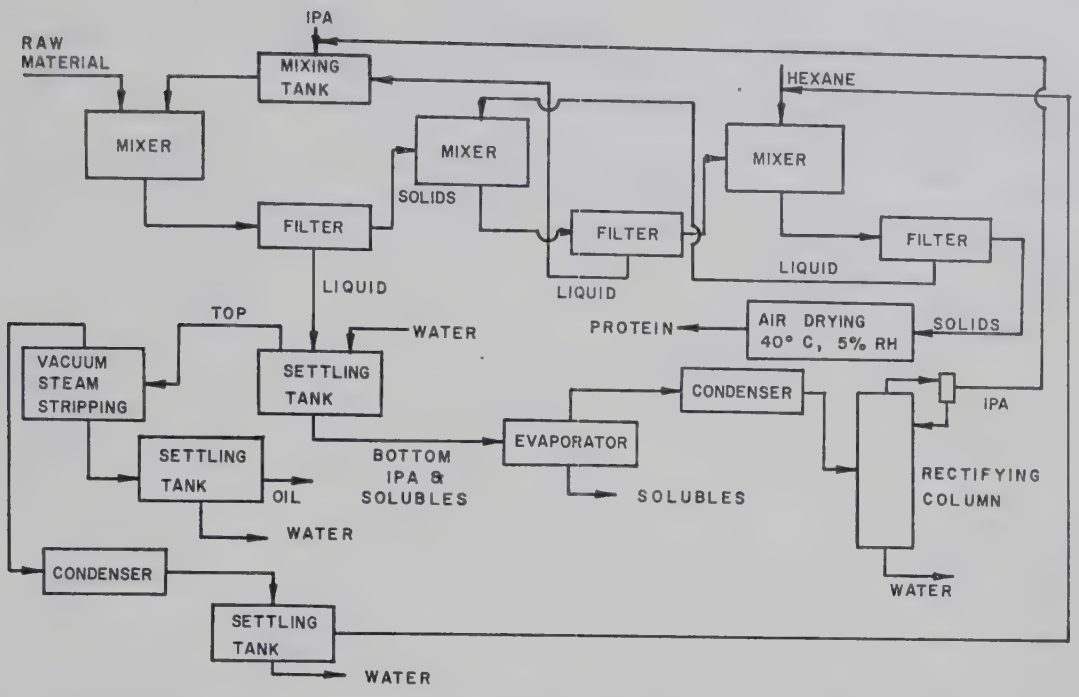


Fig. 2.–Flow diagram of protein concentrate production including solvent recovery and oil purification using IPA-Hexane as the solvent.

Recovery of the solvent and oil is based on the principle that fats or oils have negligible solubility in IPA containing more than 35 % water at 30 C (Toledo, 1973; Harris, **et al.**, 1947). Thus, when the first stage miscella is diluted with water to cause a separation of the mixture into an IPA-rich phase and a hexanerich phase, almost all of the oil will be in the latter.

Figure 3 shows a phase diagram for the system IPA-hexane-water at 25 C (Farkas, 1959). At IPA concentrations above 58 % any combination of IPA hexane and water exist as one phase. Below 58 % IPA, except for one mixture (53 % IPA, 12 % water, and 35 % hexane), all other mixtures would separate into two phases. One phase is a hexane-rich phase and the other is an aqueous IPA phase. The cross lines in the diagram are tie lines and any mixture whose composition falls along the line would separate into two phases with the composition of each phase represented by the intersection of the tie line with the phase equilibrium curve (Perry **et al.**, 1963).

The amount of water that must be added to the first stage miscella should be calculated such

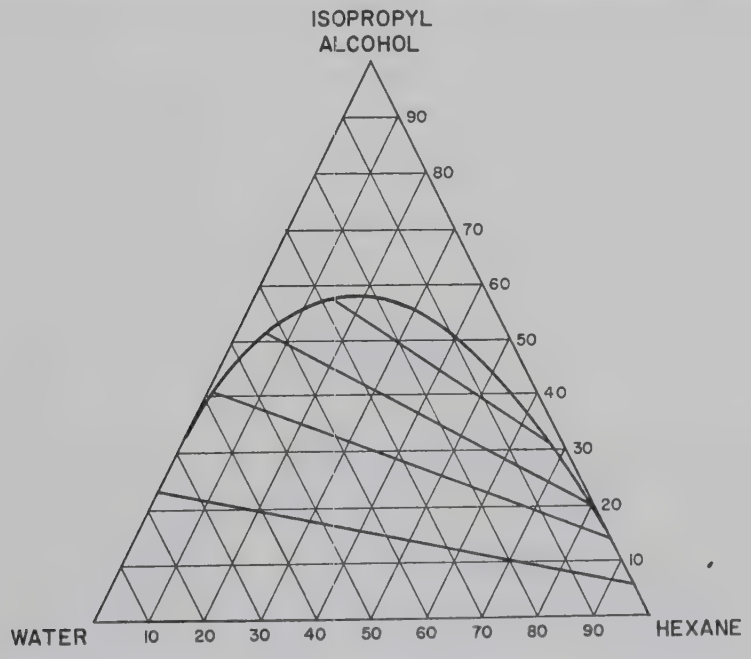


Fig. 3.–Phase diagram of the system IPA-Hexane – water at 25 C.

TABLE 1

Comparison of solvent requirements for IPA and IPA-hexane extraction at 30° C for various materials

	Proximate analysis (%)		Solvent requirement (lbs/lb raw material)			
				IPA-hexane		
Raw material	Fat	Water	85 % IPA 30° C	1st extraction IPA	1st extraction Hexane	2nd & 3rd extraction Hexane
Deboned chicken meat.	21	64	9.4	3	2	2
Beef trimmings.	71.7	17.7	18.1	0.9	3	3
Pork fat.	71	12.5	18.0	0.6	3	3
Soaked soybean.	9.4	55	5	2.6	2	2
Checken preen gland. .	39	49	9.4	1.2	2	2

TABLE 2

Stability of meat batters containing varying quantities of protein concentrate from deboned chicken meat extracted at 30° C using IPA-hexane

Formulations (9)					Stability (ml separated from 50 g plugs after 1 hour of heating at 80° C)	
Protein concentrate	Boneless beef	Pork back fat	Seasoning	Ice	Fat	Water
0	709	908	20	780	2.1	8.3
29.4	709	908	20	349	0.4	2.3
13.9	709	908	20	419	0.2	1.1

that the percentage of water, hexane, and IPA would represent a point on the phase diagram which would fall bellow the lowest tie line shown on the diagram. With this mixture, the concentration of IPA in the hexane-rich phase would be minimum and would facilitate desolventization of both protein and oil. Furthermore, the concentration of hexane in the aqueous phase would be negligible and thus allow for maximum recovery of both hexane and IPA.

Evaluation of functional properties of extracted animal proteins

The properties of protein concentrate from deboned chicken meat extracted with IPA and IPA-hexane were compared.

Wettability of the protein powder was evaluated by adding 5 grams of powder to 50 ml of water in a beaker, allowing to stand for 10 minutes, and comparing the quantity of powder that remained on the surface.

The ability of the protein powder to bind water and fat in a comminuted meat formulation, was evaluated by chopping varying amounts of the powder with a fixed amount of boneless beef and pork back fat. The moisture content of the mixture was adjusted by varying the amount of ice added. The formulation shown in Table 2 was the same as that reported by Toledo (1973). After chopping the mixture in a Hobart model 8181D chopper (Hobart Mfg. Co., Troy, Ohio), 50 gram cylindrical shaped

samples were taken, placed inside erlenmeyer flasks, and heated at 80 C for one hour in a water bath. The volumes of the fat and water that separated from the samples were measured.

The protein powder by itself was evaluated for its ability to gel and bind water and oil. 20 g of the powder was mixed with 200 ml of 4% NaCl solution at 5 C, and blended in an Osterizer model 84 T blender (John Oster Mfg. Co., Milwaukee, Wis.) at maximum speed for 5 minutes. The slurry was centrifuged at 12,000 g for 10 minutes, and the supernatant collected. To 20 ml of the supernatant, 30 ml of corn oil was added and the mixture was blended for 20 seconds in a small jar at miximum speed using the same blender above. The emulsion was transferred to 50 ml beakers and heated in a 98 C water bath for 30 minutes. The emulsion was evaluated for its ability to gel. The plugs of unsolubilized protein which remained at the bottom of the centrifuge tubes were collected and mixed with 30 ml of corn oil and blened for 1 minute in a small jar using the Osterizer blender at maximum speed. The mixture was transferred into a 50 ml beaker and packed such that a solid cylinder touching the sides of the beaker was obtained. The beaker was heated at 98 C for 30 minutes in a water bath. The cylinder was then removed from the beaker and evaluated for firmness of the gel.

Analytical procedures

Proximate analysis for fat protein and moisture was carried out using AOAC (1970) procedures for analysis of meat and meat products.

Extraction of soybeans

The use of the IPA-hexane solvent extraction system as shown in Figure 2 was evaluated for simultaneous extraction of oil and carbohydrates from the soybean. The extracted and desolventized meat analyzed for glucose, sucrose, and oligosaccharides (raffinose and stachyose) following a procedure reported by Kin **et al.** (1973).

RESULTS AND DISCUSSION

The IPA-hexane extraction process

Table 3 shows the proximate analysis of the proteins produced by solvent extraction at 24 C using the IPA-hexane extraction process. The quantities of solvent used in the three stage extraction processes are shown in Table 1. These results show that the processes and the solvent to solids ratios utilized were sufficient to produce protein concentrates with fat contents less than 1 %.

TABLE 3

Proximate analysis of protein concentrates extracted using a three-stage IPA-hexane process

	Proximate analysis in percent		
Raw material	Fat	Moisture	Protein (N X 6.25)
Deboned chicken meat.	0.3	1.8	82.9
Beef trimmings.	0.8	3.2	72.3
Pork back fat.	0.6	4.1	77.1
Chicken preen gland. ..	0.4	2,5	81.2

Preliminary testing of this solvent extraction process has shown that mixing the IPA with nexane prior to addition to the raw material in the first extraction stage allowed better fat removal compared to where IPA was added first, even if the same solids to solvent ratios were utilized. Using mixed IPA and hexane in the first stage followd by IPA in the second and third extraction stages not only resulted in a very high residual fat but also reduced in poor wettability of the dehydrated protein concentrate. These results are shown in Table 4.

TABLE 4

Effect of sequence of addition of IPA and hexane on residual fat and wettability of the protein concentrate

Treatment	Residual fat (%)	Wettability[1]
First Stage – IPA + Meat (Mix) + Hexane ..... Second & third stage – hexane	2.53	Good
First Stage – IPA + Hexane (Mix) + Meat ..... Second & third stage – hexane	0.87	Good
First Stage – IPA + Meat (Mix) + Hexane..... Second & third Stage – IPA	6.5	Poor

[1] Good wettability indicates that powder obtained after desolventization and drying absorbed water and dispersed throughout the suspending medium without stirring.

The system for solvent recovery shown in Figure 2 was tested in repeated series of extractions using the recovered solvent. In 5 repeated cycles of the solvent, there was no appreciable change in the extraction efficiency or in the quality of the desolventized protein concentrates. Make-up hexane amounted to approximately 10 % of the initial hexane input, per cycle. No attempt was made to recover the hexane that went with the solids in the last extraction stage. In the absence of a good rectifying column, IPA losses were much higher and approximately 50 % of the initial amount of IPA had to be added to each cycle. These results, however, indicate that the system for extraction and solvent recovery shown in the flow diagram in Figure 2, is technically feasible.

Comparison of solvent requirements for IPA-hexane and IPA extractions

Table 1 shows the solvent requirements for extraction of various materials with IPA or with the IPA-hexane solvent system. Because of the poor solubility of fats or oils in aqueous IPA at 30 C (Toledo, 1973), large quantities of IPA are required for extracting materials that are high in fat and moisture.

In the case of the IPA-hexane system, it has been found that with chicken fat, as high as 30 % oil can dissolve in the hexane-rich single phase mixtures of IPA hexane and water (points on or outside the hexane side of the phase equilibrium curve in Figure 3). In excess of 30 % oil, the mixtures became viscous and they did not drain readily through the filter cake during filtration. The hexane requirement for the IPA-hexane system shown in Table 1 was calculated for approximately 20 % fat in the hexane phase. Since lower quantities of solvent are required and because of the ease of recovery of hexane due to its low latent heat of vaporization, the IPA-hexane solvent extraction system would therefore allow practical use of low temperature extractions for the preparation of functional protein concentrates from animal sources.

Functional properties of low temperature extracted animal protein concentrates.

Toledo (1973) and Dubrow (1973) have shown that protein concentrates prepared by extracting fish or animal tissues with IPA at temperatures bellow 45 C, have the capacity to gel and bind fat and water in a comminuted meat formulation. This result is very similar to that reported by Toledo (1973) on protein concentrates extracted using IPA at 30 C.

Figures 4A and 4B shows the ability of the IPA-hexane extracted protein

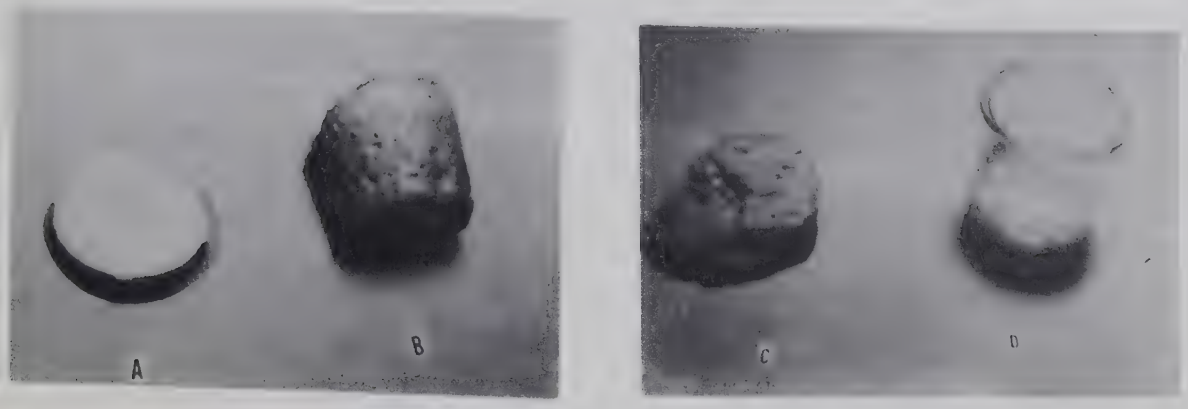


Fig. 4.–Emulsifying and gelling properties of protein concentrate (A and B are from IPA-Hexane extracted protein and C and D are from protein extracted with IPA at 50 C).

concentrate, by itself, to bind fat and water, and to gel. The solids (4A) had the ability to form a firm gel, and the salt soluble protein extract when emulsified with corn oil also gelled and bound the oil and water (4B). In contrast, non-functional protein concentrates prepared by IPA extraction at 50 C (Figures 4C and 4D) showed the solids to have very poor gelling ability (Figure 4C) and the corn oil emulsion emulsified using the salt soluble protein extract separated into an oil and water phase after heating (Figure 4D).

Wettability of the protein powder produced by IPA-hexane extraction and that produced by IPA extraction at 30 C is compared in Figures 5A and 5B. The IPA extracted protein had very poor wettability and it required vigorous mixing before it finally dispersed into the dispersion medium. Even after allowing the powder to stay in contact with water for 10 minutes followed by 1 minute of vigorous mixing with a magnetic stirrer, some of the dry powder still floated on the surface of the water. Dubrow (1973) observed the same phenomenon with IPA extracted fish proteins. He also reported that the temperature of extraction did not appear to affect the wetting characteristics of the proteins. Dubrow (1973) recommended the addition of wetting agents to the protein powder to allow for rapid wetting and easier dispersion of the powder. The IPA-hexane extracted proteins, on the other had, wetted and dispersed into the water immediately after addition, without the need for stirring (Figure 5B).

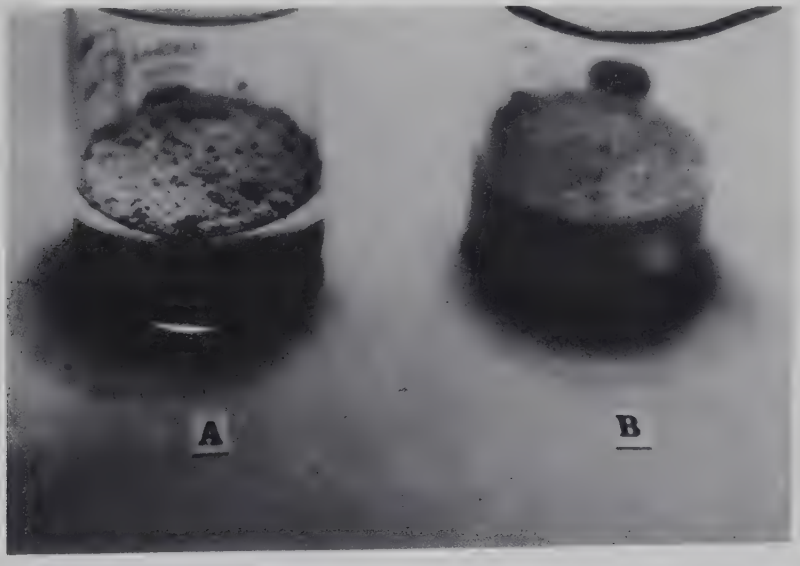


Fig. 5.–Wettability of IPA-extracted (A) and IPA-Hexane extracted (B) protein concentrate.

Use of IPA-hexane solvent extraction system on oilsedds

One disadvantage of extracting oilseeds with IPA is the capacity of aqueous IPA to dissolve carbohydrates along with the oil and thus leads to the production of a low quality dark colored oil (Harris *et al.*, 1949). Figure 6 shows soybean oil recovered from an IPA miscella (6A) and that recovered from an IPA-hexane miscella (6B). In the latter process, desolventization of the oil took less time and required less heat, compared to the IPA miscella.

The carbohydrate content of soybean meal extracted using the IPA-hexane system is shown in Table 5. The process appeared to be suitable for simultaneous extraction of oil and carbohydrates from the meal without having the serious disadvantage of a single solvent IPA extraction previously mentioned. The glucose, sucrose, and oligosaccharide content of the IPA-hexane extracted meal was much lower than that in conventional hexane extracted meal.

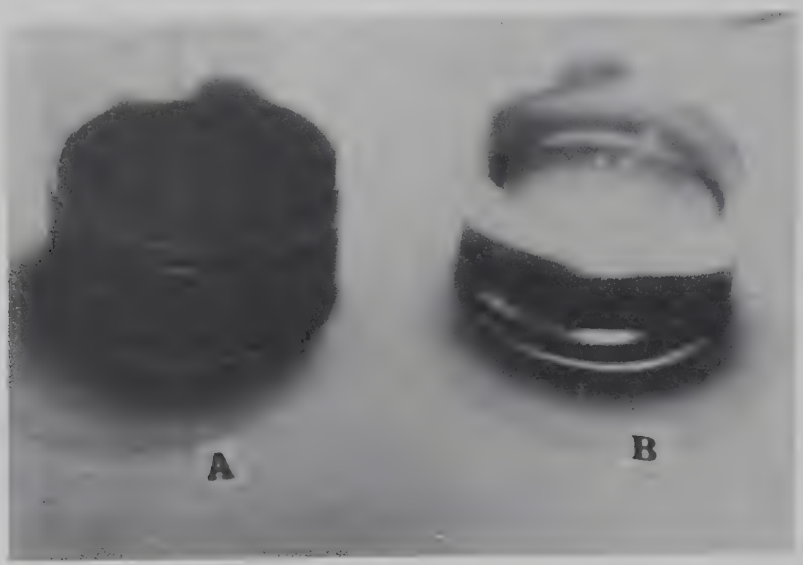


Fig. 6.–Soybean oil recovered from an IPA extraction miscella (A) and from and IPA-Hexane extraction miscella (B).

TABLE 5

Comparison of carbohydrate content of hexane and IPA-hexane extracted soybean

Treatment	Carbohydrate content mg/g dried defatted meal		
	Glucose	Sucrose	Raffinose & stacchyose
Hexane extraction			
Dry soybean.	23.2	12	30.2
IPA-hexane extraction			
Soaked soybean. . . .	3.6	5.8	4

CONCLUSIONS

The extraction process using IPA and hexane in the first stage followed by hexane in the succeeding stages, produced functional animal protein concentrates having good wettability. Solvent requirements for this process is much lower than in

IPA extractions at 24 C, thus making low temperature extractions for protein concentrate production feasible. Desolventization procedures for both oil and protein in the IPA-hexane extraction process is much simpler compared to the IPA extraction process. The IPA-hexane extraction process can also simultaneously extract oil and carbohydrates from soybeans.

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Essential aminoacids contained in by-products from the meat industry

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ABSTRACT

1) *Specific objective of this study:* enlarge the availability of plastic proteins for human consumption.

2) *Results obtained:* I.- Products with 91.5 % ± 1.79 of protein, the rest being water, mineral salts and fats, have being obtained from the small intestine of beef cattle; II.- Depending of the portion of intestine used and also on the pre-treatment of it, it was possible to obtain products that, although responding to the general composition above stated, differed however quantitatively in their aminoacid content, so that, we have designated each product differently: MPT-1, MPT-2, etc.; III.- MPT-1 showed a content of: Tryptophane (Try) 2.8 % ± 0.47; Lysine (Ly) 10.7 ± 1.7; Methionine (Me) 5.7 ± 0.99; Threonine (Thr) 5.49 ± 3.33, the figures representing grams of the respective aminoacid contained in 100 grams of MPT-1, and being the number of samples analyzed 88 for Try; 79 for Ly; 76 for Me and 57 for Thr. IV.– MPT-2 showed a Try content as high as 8.4 ± 1.59 (figures from 39 samples analyzed).

3) *Conclusions:* the small intestine of beef cattle is a rich source of Try, Ly, and Me with which it is possible to more than duplicate the plastic protein content of wheat and corn flours and other vegetable products.

INTRODUCTION

Regarding human nutrition, it is widely recognized that the biggest problem is that of finding new protein sources. But such proteins should be the so called plastic proteins, designation that in this paper means any protein containing all the essential aminoacids and besides each of them in a percentage of not less than the one established for the "Standard Protein" also called "Reference Protein" of FAO (1).

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It is worth to be noted that the literature on the subject affords very few references on the aminoacid composition of proteins from beef intestine. R. J. Block "Aminoacids Hand-book" (2) that is a comprehensive book on protein analysis (as stated on the Preface) up to approximately 1953-4 and contains data over 16 animal organs, does not include intestine.

In literature up to 1973 we found only one paper specifically on the subject of percentage of essential aminoacids in beef intestine (3), another one with values for hog intestine (4) and one for beef offals (5). It was not possible to find more information, not even in the Chemical Abstracts or the various volumes of Analytical Biochemistry (6 a, b, c, d and e) in which approximately 1000 papers are reviewed. Previous papers of the authors (3) (7) dealt with: I) the essential aminoacids content of several bovine organs; II) the procedure to obtaine concentrated and in a powdered form, the proteic fraction rich in those essential aminoacids; III) the different ways which would permit to supplement with those fractions, foods of vegetable origin such as for instance, wheat and corn flours, rice, etc. IV) results obtained by a taste panel which after texting dishes prepared with the enriched flours and rice, concluded that the enrichment did not change the organoleptic characteristics of the respective foods.

However, as stated in a previous report refering to the essential aminoacids content of several bovine organs and on account of the few experiences carried out until the date of that report, the work was to be completed with a larger number of determinations. The authors continued work, focusing their attention specially on the small intestine, firstly because of the high content of Try, Ly and Me found in it and secondly, because this organ, some years ago almost completely used as casing in the sausage manufacture, is to day more and more being replaced by the so called synthetic collagen casings.

In this paper the authors give the results obtained after analysing 127 samples for Try, 79 for Ly, 76 for Me, 57 for Thr, 31 for Beta-phenilalanine, 31 for Leucine, 31 for Isoleucine and 40 samples for Valine, all samples having being obtained from the bovine small intestine. With the aim at obtaining the main objective of these studies, taht is to say to enlarge the availability of plastic proteins for human consumption, the particular objective in this case is to determine the richness of beef cattle small intestine in the essential aminoacids, specially the richness in Try, Ly and Me and consequently, to know if this organ can be used as source with which it is possible to supplement vegetable foods.

RESULTS AND DISCUSSION

I) The analysis of 19 samples of the powdered product obtained from the small intestine of beef cattle revealed a protein content of 91.5 % ± 0.79, the rest being water, mineral salts and fats. The authors consider that although not very large the number of analyzed samples, 19, this number is quite enough however, on account of (a): the values of the essential aminoacids content is given as percentages in the proteic fraction and (b) the differences in protein content between the analyzed samples were rather small

II) Depending on the portion of intestine used and also on the pre-treatment of it, it was possible to obtain products that, although responding to the general composition above mentioned, differed, however, quantitatively in their aminoacid content so that the authors have designated each product differently as MPT-1, MPT-2 atc.

III) MPT-1 showed a content of: Try 2,8 ±0.47; Ly 10,7 ±0.99; Thr 5.49 ± 3.33; Me 5.7 ± 0.99, the figures representing grams of the respective aminoacid contained in 100 grams of MPT-1, and being the number of samples analyzed 88 for Try, 79 for Ly, 76 for Me and 57 for Thr.

IV) With respect to the essential aminoacids Beta-phenilalanine, Leucine, Isoleucine and Valine, in all cases the lowest value registered in the analysis of samples of MPT-1 was higher than the respective values stated for FAO's "Reference Protein" and besides, wheat and corn flours and rice have also higher values than those of the mentioned "Reference Protein".

Therefore, only in the case of Thr some difficulty may be encountered when trying to enrich wheat flor with MPT-1, since the percentage of this aminoacid in wheat flour protein is approximately the same established by the Standard Protein, and, although the average value for this aminoacid in the MPT-1 is rather high (5.49 %) the standard deviation is too large. It is then evident that the analyzed samples were not homogeneous, and this is true not only for Thr but also for the other essential aminoacids considered, that is why the authors will continue the work trying to determine which factors are responsible for the heterogeinity of the results thinking that the main reason may be either that the different portions of the small intestine contain different quantities of each aminoacid, either that those quantities are related to the pre-treatment suffered by the raw material used to obtain MPT-1.

V) MPT-2 showed a Try content as high as 8.4 % ±1.59, result obtained from analysis of 39 samples. In this last material (MPT-2) the other aminoacids have not yet being determined.

CONCLUSIONS

I) The small intestine of beef cattle is a rich source of those essential aminoacids in which vegetable foods are defficient, specially a rich source of those essential aminoacis in which vegetable foods are defficient, specially a rich source of Try, Ly and Me.

II) By a rather simple procedure, it is possible to obtain from the small intestine of beef cattle the proteic fraction which contains the above mentioned aminoacids.

III) It is possible to more than duplicate the plastic protein content of wheat and corn flours without altering the organoleptic characteristics of the foods prepared with the enriched flours.

IV) The authors consider that many other determinations must be carried out, such as for instance: a) to determine the average value of the total quantity of each essential aminoacid which is possible to recover from each animal; b) the quantitative analysis of total sulphur containing aminoacids; c) the quantitative analysis of total aromatic aminoacids; d) to test biologically the true value of the enriched foods; e) others.

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Session 8a
Summary and Conclusions

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The problems connected with the solving of the nutrition of the rapidly growing mankind are of very complex character. The complexity of problems is also reflected in the papers read at the sessions of topic 8a: New food sources of key nutrients. Moreover on the basis of papers and discussion it is possible to make some general conclusions.

It is doubtless that one of the possibilities for growing the food production may be the exploration of new food sources. On the basis of investigations of last decades and some tendencies of food production it can be stated -which is also reflected by papers presented in this session- that the potential new food sources of key nutrients may be the following ones:

— oil seeds (oil seeds protein)
— at this time neglected cereals and other plant seeds
— green parts of different plants at time not used for human nutrition
— byproducts of milk and meat industries.
— food sources of microbiological origin
— algae

The use of the oil seeds protein directly in the human nutrition has a growing tendency (texturized food proteins, high protein beverages, cereal products enriched with oil seeds protein, etc.). Moreover not all possibilities are investigated.

The results of investigations presented in the papers show the possibility of use some very promising new cereals (for example in India) in human nutrition and also such well known seeds as different varieties of bean, peas, etc.

The leaves of many vegetables are widely used as human foods. The wide variability of green plants and a big quantity of production of different green plant parts give many potential possibilities to increase directly the sources of key nutrients. A good example of them is the production of leaf proteins and investigations for use them in human production or the investigation of some indian green plants having an extraordinary high content in vitamins and minerals.

Among the byproducts of milk industry the production of different whey protein concentrates is very promising in extending of protein sources.

The production of single cell proteins by and industrial may open also great possibilities. Moreover many basic nutritional problems (for example: high nucleic acid content of yeasts, green-blue color of algae products, etc.) are not solved.

A further investigation of chemical composition and biological value of potential new food sources, the solving of technological problems of production in an economical way of new food sources is very important and may be all side supported.

Session 8b

EXPLOITING LOCAL FOOD RAW MATERIALS

Chaired by

A. Gorgatti, Brazil and P. C. Spensley, United Kingdom

Secretary

Miguel Rodrigo Enguídanos, Spain

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STATUS REPORT

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I am greatly honoured to have the duty, in this important Congress, of chairing this Topic, jointly with Dr Gorgatti. After the opening session this morning, with its compelling statements by Prof. Stewart and Prof. Scrimshaw, we will all, I'm sure, be more conscious than ever of the importance of the subject matter that Topic 8b is concerned with. The fact that we have grown to three meetings —today's and the two extraordinary sesions tomorrow— is further evidence of the extent of interest in this area.

1. The title of Topic 8b is "Exploiting local food raw materials" and it clearly has the needs and problems of developing countries particularly in mind. I think we may assume also that the section was meant mainly to embrace exploitation for local consumption and not production and preparation for export from the locality or country, though we have at least one paper in the meetings now which is to do with a commodity for export. I think we can also take it that we are concerned mainly with exploitation which is aimed at improving the nutritional status of the locality and which, therefore, adds to the quantity, quality, variety or convenience of the dietary components available.

2. There are, no doubt, several ways in which one could sub-divide this subject area. In this brief introduction I suggest we think of a breakdown in terms of 5 major objectives:

(I) The preparation of special, processed and composite foods for the nutritionally vulnerable groups in the population (also ingredients for these).
(II) The improvement in quality, attractiveness and availability of existing foods used by the population at large.
(III) Easing the preparation, and the development of convenience forms of existing foods.
(IV) The development of novel foods from locally available materials.
(V) The saving of imports by the use of local foodstuffs.

3. It is not, I think, a main aim of this Session to consider the use of local materials to produce animal feeds, even though, in turn, these may provide nutritious and acceptable foods for human consumption. However, there is often an inter-relationship between the two types of usage. Several products first established as

animal feeds can, with refined processing, be used —or be contemplated for use— for direct human feeding. One or two papers in this Session are in this area.

4. I will now attempt a brief comment on these five objectives. I would emphasise, however, that many projects and developments are directed to more than one of the objectives. Papers in this Session illustrate this.

5. Under the first objective —the preparation of special foods for the vulnerable groups— the aspect of greatest importance is, no doubt, the matter of special foods for infants and young children. Much has been said and written in recent years about "the world protein crisis", but the view is now taking hold that a large part of the problem should be regarded as an overall food shortage rather than a lack of protein. (1) However, the group where true and serious protein deficiency is most liable to occur is the infant and young child section of the community, as this requires a higher percentage of protein in the diet for healthy growth.

6. Foods for this group have received a great deal of attention in many quarters in recent years; but there has also been difficulty and frustration for those involved, this stemming basically from the fact that those most in need tend also to be those who are least accessible and are least able to pay for specially processed and compounded products. Miss Elizabeth Orr, at TPI, has reviewed this whole matter in a report prepared for the Protein Advisory Group of the United Nations System (2) and has concluded that additional reasons for the failure of particular protein-rich food products have been unacceptability, failure to promote satisfactorily and lack of institutional markets. The contribution made so far by the manufactured protein-rich foods, is very small in world terms. Nonetheless, experience has proved that products of high nutritive value can be made from vegetable materials using existing technology; that these products can be acceptable and that they can be considerably cheaper than many established, processed foods intended for vulnerable group use.

7. There is really no shortage in world terms of suitable protein-rich ingredients for use in special foods for infants and young children. Existing production of oilseeds could provide the main protein component of all the supplementary foods presently required and this source has the merit that it gives a product which is readily prepared and is easily and acceptably incorporated into special foods. (3) Their cost of production is also generally lower than that for alternative materials. While this source is so under-utilized, efforts to develop and promote less suitable protein-rich components need careful appraisal.

8. Certain oilseeds, such as groundnuts and coconuts, are already traditionally used as foods with little or no processing. Sesame seeds are used as ingredients of certain confections consumed particularly in countries of South East Europe and the Middle East. Soya beans are also consumed in large quantities in countries of the Far East, after various traditional methods of processing. Nonetheless, only a small proportion of the oilseed protein produced in the world today contributes directly to human nutrition, most of the residues after oil extraction being used for animal feeding. Such products of oil extraction are not normally produced to standards suitable for human consumption and in some instances toxic or anti-nutritional factors are present. Modified production methods are therefore called for, and considerable attention has been directed to this end in recent years.

9. This first objective, bearing in mind that it embraces possible ingredients for special, vulnerable group foods, is covered by the largest number of papers. The one by Miss Cameron and Dr Hofvander was specially invited. You may care to note that the 8A sessions also contain a number of papers in this general area.

10. The second objective —the improvement in quality, attractiveness and availability of existing foods used by the population at large— embraces a variety of aspects. I would like to single out as perhaps the most important of these, that of the preservation of foodstuffs in good condition from the time of production until they are required for consumption.

The need to preserve and store may be the basic one of holding staple materials from harvest, for anything up to a year, until the next harvest has arrived; or it may be required to keep material in good condition whilst it is transported to a more or less distant point for consumption.

11. In the first of these categories comes the matter of farmer or village storage of grain, legumes and root crops, and this has been receiving a fair amount of attention of late, though this Congress seems to have no paper on the subject. Much, indeed, has been said, quoted and requoted about the extent of storage losses of staple food in developing countries, but little really reliable data is available. Current work at TPI, the University of Sussex and elsewhere to try and determine practical losses and to assess their economic significance, should help provide a more reliable base than has been available hitherto for estimating the value of introducing improved storage techniques.

12. I mentioned root crops just now. These are the staple food for 400-500 million people in the tropics. Amongst them is cassava, with its high yield potential. Its value is however, limited by its poor storage life. Current work to overcome this is, therefore, very significant. There is also scope for new methods of processing and for greater industrialisation of cassava and of many other crops.

13. Meat and fish, though valuable nutritionally, tend to be expensive and, in poorer areas, may mainly feature as a condiment or relish rather than as a main foodstuff. Such strongly flavoured products as smoke-dried meats and fish sauces thus come to be used in diets that otherwise might be bland and monotonous. It may sometimes be a mistake, therefore, to seek to produce a flavourless, odourless product such as fish protein concentrate. Indian experiments have recently aimed at producing a product resembling fish meal but having a desirable "fishy" flavour. Fish sauces, made by a fermentative process, are popular in some areas but traditional methods of manufacture may occupy a year or more. Not surprisingly, therefore, research is in hand in one or two places with the aim of producing equally acceptable products more quickly and simply.

14. The invited paper, contributing to this second objective is by Dr Jones on the aspect of preservation of perishable material for wider distribution, in this case fish. We will hear this shortly. Papers, on the preparation and preservation of coconut products we will hear soon.

15. The third objective —easing the preparation and the development of convenience forms of existing foods— has, as a main feature, the mechanisation of production of **traditional** foods. Traditional production methods are, in many instances, very time-consuming and laborious and there is a growing need and demand for the saving of labour, not only in order to improve the quality of life directly, but also to allow more time and effort to be devoted to gainful activities. The mechanisation can, however, be at various levels of sophistication, ranging from simple aids to hand processing, to fully automatic, factory scale operations. The best level of technology to adopt in any particular instance —what we in TPI call the "Optimal Technology"— may be at either end of the scale, or in the middle, and needs to be determined by a careful assessment of all the technological, economic,

sociological and political factors, relevant in each case. Rigid adherence to particular, broadlybased economic or sociological theories is not, in my view, helpful.

16. At the simple end of food technology scale, the kind of processes that still seem to offer possibilities for development are operations such as decortication, milling and oil extration for use at the village level. Where it is necessary still to think in terms of man-powered processes, attention may be directed —and is being directed in several recent projects— to pedal-powered machines, since, in general, an operator can sustain greater energy inputs this way. Important also is the improvement of food preservation methods suited to the scale of village operations— moisture reduction, salting, smoking and fermentative operations, for instance.

17. We have several papers on the modernisation of production of traditional foods.

18. The fourth objective —the development of novel foods from locally available materials— is certainly an area with a future, offering scope for investigation and ingenuity. I was glad to find that there were two interesting contributions to the session which fall into this category. The papers by Deethardt et al and Payumo et al will be given in this area.

19. I would merely add regarding this objective, that the textured vegetable proteins, in particular, seem destined for a world-wide role, especially when it is remembered —as Prof. Scrimshaw emphasised at the opening session— that their production involves so much less land per unit of nutritional value compared with the animal proteins which they can simulate.

20. The fifth and final objective is concerned with saving of imports by the use of local foodstuffs; and of particular interest here is the idea of using locally available starches in composites flours for making bread and other bakery products. Because of the leading role that FAO has played in stimulating work on this subject, the third invited paper for this session in provided by that organisation — and we will hear this shortly. It is convenient also to consider in this group, the fortitication of bakery and wheat products generally. This gives us a group of 4 papers. On composite flour, I would merely emphasise that it is the economics of its use that now presents the major question. This is under investigation.

21. In conclusion, I should perhaps mention a general matter, bearing to some extent on all the five objectives — that is the matter of national food and nutrition planning. Experience particularly with the processed foods for vulnerable groups in developing countries has revealed the vital importance of thorough planning. Efforts presently being made to help with the establishment of national bodies for the planning and implementation of food and nutrition policies are, surely, most significant.

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Production and marketing of composite flour bakery goods in developing countries

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ABSTRACT

Many developing countries in the tropics and subtropics have become increasingly dependent on imported cereals to supply their urban population with basic foodstuffs. Heavy purchases of wheat from temperate zone industrialized countries, obtained with hard currencies at highly competitive world market prices have caused severe strains on some national economies. For ten years FAO has been actively engaged in a programme of cooperation with several of the world's leading cereal research laboratories to develop bakery products from mixtures containing significant proportions of flours, of non-wheat origin, that can be processed in the developing countries from their own indigenous crops.

Within the last five years projects have been in operation in Africa, Asia and Latin America to determine consumer acceptance of some of the products formulated from blends of imported and local raw materials. Market tests conducted for periods of more than twelve months have proven that many consumers will accept bakery products made from composite flours provided that their quality, cost and presentation are satisfactory.

INTRODUCTION

The following description has been used within FAO as a definition of "Composite Flours" since a programme of research and development was initiated by the Organization in 1964:

The term is employed to describe all flour mixtures formulated for the production of bakery, confectionary and pasta goods that contain significant proportions of: (1) cereal flours other that wheat, (2) flours and starches from roots, tubers, pulses, etc., (3) protein concentrates from oilseeds, fish, yeasts, etc., blended with wheat flour, and (4) combinations of these materials but excluding wheat flour as an ingredient. In the preparation of the products it is considered necessary to incorporate additives, apply further processing to certain of the ingredients, or employ mixing techniques specially developed, in order to achieve volume and/or textural properties acceptable to consumers.

TABLE 1

*Imports of Wheat and Flour 1971-73**
(Calendar years: Jan/Dec) –flour in wheat equivalent–

Country	Quantity/Metric Tons			Value/US$		
	1971	1973	Variation	1971	1973	Variation
(a) AFRICA						
Non-wheat producers						
Cameroon.	67,788	73,180	+ 5,392	4,330,000	7,200,000(F)	+ 2,870,000
Cent. Afr. Rep.	14,467	15.723	+ 1,256	1,250,000	1,800,000	+ 550,000
Congo.	32,286*	32,557*	+ 271	2,300,000*	4,000,000*	+ 1,700,000
Dahomey[+].	16,494	9,167	– 7,327	1,154,000	1,120,000	– 34,000
Gabon.	8,334 (F)	8,890 (F)	+ 556	750,000(F)	1,000,000(F)	+ 250,000
Gambia.	3,046	2,917	– 129	286,000	300,000	+ 14,000
Ghana.	46,642	100,195 (F)	+ 53,553	3,655,000	12,440,000(F)	+ 8,785,000
Guinea[+].	16,668 (F)	25,002*	+ 8,334	1,100,000(F)	2,700,000(F)	+ 1,600,000
Ivory Coast.	32,205	141,806	+ 109,601	2,833,000	18,210,000	+15,377,000
Liberia.	9,877	8,634	– 1,243	1,498,000	1,080,000	– 418,000
Madagascar.	38,491	30,583	– 7,908	2,853,000	3,008,000	+ 155,000
Malawi[+].	27,897	28,317 (F)	+ 420	2,639,000	3,415,000(F)	+ 776,000
Mali[+].	16,562	20,835	+ 4,273	1,487,000	2,400,000	+ 913,000
Mauritania.	16,668 (F)	18,335 (F)	+ 1,667	1,300,000(F)	1,800,000(F)	+ 500,000
Mauritius.	60,491	72,170 (F)	+ 11,679	4,281,000	7,305,000(F)	+ 3,024,000
Niger[+].	4,849	5,556*	+ 707	470,000	700,000*	+ 230,000
Nigeria.	405,053	413,890	+ 8,837	33,571,000	51,600,000	+18,029,000

Rwanda[+]	5,551	5,278 (F)	− 273	403,000	560,000(F)	+ 157,000
Senegal	113,452	148,167*	+ 34,715	9,449,000	13,500,000*	+ 4,051,000
Sierra Leone	31,043	37,556	+ 6,513	2,962,000	5,450,000	+ 2,488,000
Somalia	17,804	71,539 (F)	+ 53,735	1,170,000(F)	6,770,000(F)	+ 5,600,000
Togo	14,026	12,501*	− 1,525	1,103,000	1,800,000(F)	+ 697,000
Uganda[+]	19,458	55,600	+ 36,142	1,662,000	6,100,000	+ 4,438,000
Upper Volta[+]	27,612*	25,389	− 2,223	2,150,000*	2,540,000	+ 390,000
Zaire	111,120 (F)	110,642	− 478	9,000,000(F)	11,284,000	+ 2,284,000
Zambia	82,587	112,084 (F)	+ 29,497	6,269,000	11,200,000	+ 4,931,000
Wheat producers						
Algeria	698,400	904,725*	+ 206,325	48,600,000	93,200,000	+44,600,000
Angola	87,403	73,890(F)	− 13,513	6,888,000	7,000,000(F)	+ 112,000
Burundi[+]	10,409	4,756	− 5,653	984,000	606,000	− 378,000
Chad[+]	11,306	16,167	+ 4,861	2,092,000	3,050,000	+ 958,000
Egypt	2,490,274	1,805,182	− 604,092	159,972,000	165,141,000	+ 5,169,000
Ethiopia	45,805	12,000	− 33,805	3,638,000	1,400,000(F)	− 2,238,000
Kenya	16,395	47,639	+ 31,244	1,160,000	3,840,000 (F)	+ 2,680,000
Lesotho[+]	39,257*	55,560(F)	+ 16,303	12,000,000(F)	16,000,000(F)	+ 4,000,000
Libyan Arab. Rep.	243,790	337,800	+ 94,010	15,852,000	29,000,000(F)	+13,148,000
Morocco	756,968	967,441	+ 210,473	54,790,000	120,300,000	+65,510,000
Mozambique	87,104	105,556	+ 18,452	6,271,000	11,500,000	+ 5,229,000
Rhodesia	87,278	92,506(F)	+ 5,228	5,625,000	8,327,000(F)	+ 2,702,000
Sudan[+]	185,359	142,410	− 42,949	14,236,000	14,569,000*	+ 330,000
Tanzania[+]	24,300	42,630(F)	+ 18,330	1,730,000	3,400,000(F)	+ 1,670,000
Tunisia	256,777	226,625	− 30,152	18,749,000	28,297,000	+ 9,548,000

*Developing country only

(b) ASIA AND THE FAR EAST

Non-wheat producers

Bhutan[+]	1,994(F)	2,074(F)	+ 80	271,000(F)	282,000(F)	+ 11,000
Brunei	3,006	3,056(F)	+ 50	239,000	295,000(F)	+ 56,000
Hong Kong	170,331	158,687	— 11,644	12,115,000	19,731,000	+ 7,616,000
Indonesia	712,987	899,771*	+ 186,784	51,200,000(F)	112,700,000(F)	+61,500,000
Khmer Rep.	34,725	41,245	+ 6,520	2,200,000	4,265,000	+ 2,065,000
Laos[+]	4,489	7,251(F)	+ 2,762	400,000	1,040,000(F)	+ 640,000
Malaysia	327,922	419,122(F)	+ 91,200	23,625,000	44,036,00(F)	+20,411,000
Philippines	611,159	531,748	— 79,411	38,353,000	51,997,000	+13,644,000
Sikkim[+]	3,010(F)	3,150(F)	+ 140	439,000(F)	460,000(F)	+ 21,000
Singapore	178,976	239,783	+ 60,807	12,270,000	28,045,000	+15,775,000
Sri-Lanka	492,553	660,757	+ 168,204	38,599,000	76,732,000	+38,133,000
Thailand	63,706	96,945*	+ 33,240	5,317,000	11,700,000(F)	+ 6,383,000
Vietnam, Dem. Rep.	418,506*	349,056*	— 69,450	31,000,000	26,000,000*	— 5,000,000
Vietnam Rep.	249,937	204,725*	— 45,212	18,762,000*	22,100,000*	+ 3,338,000

Wheat producers

Bangladesh	816.525	2.520.835	+1,704,310	66,000,000	232,400,000	+166,400,000
Burma	42,481	13,890	— 28,591	3,840,000	1,300,000	— 2,540,000
China	3,918,214(F)	6,138,778(F)	+2,220,564	291,214,000(F)	640,300,000(F)	+349,086,000
India	1,916,078	3,601,667	+1,685,589	157,254,000	420,220,000	+262,966,000
Korea Dem. Rep.	300,000	871,710*	+ 571,710	23,000,000	67,000,000*	+44,000,000
Korea Rep.	1,825,918	1,748,672(F)	— 77,246	125,230,000	219,550,000(F)	—94,320,000
Mongolia	22,363	93,113*	+ 70,750	1,800,000	7,700,000*	+ 5,900,000
Nepal	265	264	—	30,000	30,000	—
Pakistan	322,211	1,368,820	+1,046,609	15,276,000	106,110,000	+90,834,000

(c) NEAR EAST

Non-wheat producers

Bahrain	17,439	8,675	− 8,764	1,409,000	1,874,000	+ 465,000
Kuwait	111,654	77,708	− 33,946	8,221,000	8,003,000	− 218,000
Qatar	14,722	14,323	− 399	1,333,000	2,680,000	+ 1,347,000
Yemen Democratic	64,027	73,615	+ 9,588	4,783,000	6,900,000	+ 2,117,000

Wheat producers

Afghanistan	485,723*	77,780(F)	− 407,943	35,762,000*	7,900,000(F)	−27,862,000
Cyprus	36,821	99,429	+ 62,608	2,640,000	15,914,000	+13,274,000
Iran	999,553	791,167	− 208,386	76,430,000	69,636,000	− 6,794,000
Iraq	955,008	154,184	− 800,824	75,202,000	21,550,000	−53,652,000
Lebanon	326,369	354,725(F)	+ 28,356	22,469,000	31,700,000(F)	+ 9,231,000
Saudi Arabia	313,348	411,010	+ 97,662	25,610,000	40,000,000	+14,390,000
Syrian Arab. Rep.	748,754	118,593	− 630,161	56,537,000	13,299,000	−43,238,000
Turkey	697,266	28,881	− 668,385	50,985,000	3,564,000	−47,421,000
Yemen Arab. Rep.	105,259*	118,283	+ 13,024	6,558,000*	13,552,000	+ 6,994,000

(d) LATIN AMERICA

Non-wheat producers

Antigua	4,167	4,167	−	400,000	450,000	+ 50,000
Bahamas	7,742	7,640(F)	− 102	960,000	1,700,000(F)	+ 740,000
Barbados	19.089	20,880(F)	+ 1,791	1,815,000	2,056,000(F)	+ 241,000
Belize	9,723(F)	10,418	+ 695	830,000(F)	1,100,000(F)	+ 270,000
Bermuda	2,778(F)	3,473(F)	+ 695	287,000	320,000	+ 33,000
Costa Rica	67,382	80,972*	+ 13,590	5,588,000	10,195,000	+ 4,607,000
Cuba	881,758	886,704*	+ 4,946	69,319,000	75,000,000*	+ 5,681,000
Dominican Rep.	97,973*	121,112	+ 23,139	7,270,000*	14,300,000	+ 7,030,000
El salvador	65,545	72,473	+ 6,928	4,780,000	9,370,000	+ 4,590,000
Fr. Guiana	3,991	4,167(F)	+ 176	515,000	650,000(F)	+ 135,000

Grenada.	7,778	8,056	+ 278	675,000	900,000	+ 225,000
Guadeloupe.	27,958	27,780(F)	— 178	3,216,000	4,400,000(F)	+ 1,184,000
Guyana.	39,629	54,167	+ 14,538	4,158,000	7,000,000(F)	+ 2,842,000
Haiti.	66,667	95,279	+ 28,612	5,130,000	11,900,000	+ 6,770,000
Honduras.	49,233	43,367	— 5,866	3,991,000	4,700,000	+ 709,000
Jamaica.	189,010(F)	209,684	+ 20,674	13,200,000(F)	22,819,000	+ 9,619,000
Martinique.	34,200	37,503(F)	+ 3,303	3,798,000	5,500,000(F)	+ 1,702,000
Neth. Antilles.	13,611	18,057(F)	+ 4,446	1,092,000	2,000,000(F)	+ 908,000
Nicaragua.	44,105	39,578	— 4,527	3,605,000	4,570,000	+ 965,000
Panamá.	55,356	45,000	— 10,356	3,857,000	6,100,000	+ 2,243,000
Surinam.	20,363	23,884	+ 3,521	1,751,000	3,002,000	+ 1,251,000
Trinidad and Tobago.	91,831	97,558	+ 5,727	7,257,000	11,400,000	+ 4,143,000
Venezuela.	596,171	540,006	— 56,165	39,974,000	68,002,000	+28,028,000
Wheat producers						
Bolivia.	173,623*	179,179	+ 5,556	14,700,000(F)	17,200,000	+ 2,500,000
Brazil.	1,739,164	3,014,471	+1,275,307	127,220,000	301,889,000	+174,669,000
Chile.	381,782	936,473*	+ 554,691	26,904,000	75,100,000	+48,196,000
Colombia.	445,842	401,064	— 44,778	29,721,000	51,238,000	+21,517,000
Ecuador.	92,905*	124,817	+ 31,912	8,000,000*	14,870,000(F)	+ 6,870,000
Guatemala.	65,683	74,917*	+ 9,234	4,345,000	7,800,000(F)	+ 3,455,000
Mexico.	177,505	718,464	+ 540,959	11,801,000	78,132,000	+66,331,000
Paraguay.	63,798	95,084	+ 31,286	3,804,000	9,940,000(F)	+ 6,136,000
Peru.	704,007	753,196	+ 49,189	52,800,000	82,176,000	+29,376,000
Uruguay.	119,000*	150,000*	+ 31,000	8,100,000(F)	17,500,000(F)	+ 9,400,000

Notes: + **Least Developed Countries (LDCs). (F) FAO estimate. * Unofficial data. — Negligible.**
Source: Trade Yearbook, Vol. 27, 1973. FAO, Rome.

OBJECTIVES

The objectives of the programme are to:

(1) collect data on the cereal industries, products manufactured, and trends in consumption of bakery goods in wheat-importing developing countries;
(2) identify local and alternative raw materials that have potential for processing and utilization in standard wheat-based products, through cooperation with national research laboratories;
(3) foster the exchange of information, and when necessary promote research to solve specific technological problems encountered in milling cereal grains other than wheat, and in the production of bread, biscuits, and pasta goods, from composites of two or more flours;
(4) assist governments of developing countries to establish joint projects sponsored by the United Nations Development Programme, or supported under bilateral aid agreements, for the purpose of conducting market tests and pre-investment feasibility studies;
(5) improve the quality, variety, and where needed the nutritional status of the local bakery products, through the use of blended flours, when this can be undertaken without raising product cost;
(6) stimulate national economies through the creation of an internal demand for substantial quantities of local grains, starches or oilseeds, where the potential for supply exists, and thereby enable savings in foreign exchange to be made, provide employment for more people in rural areas, and increase the total availability of food.

TABLE 2

Export Prices of Wheat 1972-74 – Quaterly Averages

Quarters	US No. 2 Hard Winter (Ordinary)	Canada Manitoba No. 2 & CWRS 14 %, 13.5 %	Canada No. 1 Western Amber Durum
		US$ per Metric Ton	
1972 1st.	59.95	61,09	57.70
2nd.	60.13	63.39	62.69
3rd.	67.24	69.89	70.56
4th.	93.95	89.46	94.51
1973 1st.	102.20	98.62	103.64
2nd.	104.82	106.88	113.84
3rd.	160.34	177.49	270.40
4th.	187.95	204.99	329.82
1974 1st.	208.28	217.74	307.76

Notes:

US No. 2 Hard Winter (Ordinary) f.o.b. vessel, Gulf Ports.
Canada Manitoba Northern up to July 1972, Canada Western Red Spring No. 1 14 %, up to Sept. 1973: Canada Western Red Spring No. 1 13.5 % to date f.o.b., Thunder Bay. Canada No. 1, Western Amber Durum, f.o.b. Thunder Bay.

Source: Developments and Trends in World Grain Prices. CCP:GR 74/9 July 1974, FAO, Rome (updated).

RESULTS AND DISCUSSION

Experience gained in projects on three continents, in which FAO has participated, has shown that 30 % replacement of wheat flour is the level most readily accepted at the present time. In Brazil the incorporation of 20 % - 40 % pre-gelatinized corn flour in the manufacture of noodles, crackers and cakes, has already been introduced into some commercial plants. Market tests conducted in Bogota, Colombia during 1972 indicated that bread rolls made from 70 % wheat, 27 % local rice flour, and 3 % soya, could be sold without difficulty. The additive calcium stearoyl-2-lactylate was used in this mixture. More recently, French-type bread containing 30 % millet flour, milled from one of Africa's major cereals, is now being introduced by commercial bakeries in Dakar, Senegal. It is essential to employ separate mixing of the two doughs initially to gain acceptable volume in the final product. Darker colour of loaf and different taste as well as better preservation, are factors appreciated by many consumers. Current research is being undertaken with sorghum flour in Khartoum, The Sudan, for utilization in Arabic and European-type tin bread.

Many different mixtures have been formulated in laboratories in the United States containing high levels of soya flours for use in overseas feeding programmes. Other blends utilizing cassava and soya have been developed in the United Kingdom by the Tropical Products Institute (TPI) for introduction in Africa and Asia. A significant number of research workers around the world have also been experimenting with a wide range of materials which include flours from: barley, quinoa, potato, sweet potato, taro, chickpeas, cowpeas, mung beans and protein concentrates from: peanuts, coconut and sunflower seed.

CONCLUSIONS

Provided good quality control was maintained, approximately one third replacement of wheat flour in many products presented no major difficulties in production or marketing. The primary restraint inhibiting wider commercialization is the limited availability of industrially processed flours at low enough cost to justify replacement on economic grounds. Expansion will be dependent in many countries on government support to encourage production of the crops required for processing, and to ensure that cultivators are guaranteed a satisfactory price. The cooperation of the milling and baking industry is also essential as the processing of new raw materials requires additional investment in equipment, and in the bakeries, changes in methods that involve more handling and higher costs of production. It has to be ensured that the cost of local materials is significantly lower

TABLE 3

Ocean Freight Rates for Wheat to India (East Coast) per Metric Ton

Ports of Shipment From:	1971/72 (July/June)	1972/73 (July/June)	1974 (20 August)
US Gulf Ports (Via the Cape).	US$ 10.49	US$ 17.26	US$ 52.16
North Pacific.	US$ 9.71	US$ 16.39	US$ 48.35

Source: Schedule of freight rates issued by the Secretariat, International Wheat Council, 28 Haymarket, London SW1Y 4SS.

TABLE 4

Raw Materials evaluated in baking studies, conducted in The Netherlands, sponsored by FAO 1964-70

Product	Country of origin
Cereals	
Maize flour, and starch	Brazil*, Colombia
Millet flour	Niger*, Senegal*
Sorghum flour	Sudan
Roots, Tubers, etc.	
Cassava flour, and starch	Ghana, Madagascar*, Togo*, China*, India*, Philippines*, Thailand*, Brazil*, Colombia*, Paraguay*
Sago Starch	Malaysia*
Sweet potato flour, and starch	India, Japan*
Taro flour	Hawaii
Yam flour	Nigeria
Oilseed flours, etc.	
Coconut protein concentrate	Philippines
Cottonseed flour	USA*
Groundnut (Peanut) flour	India*, Nigeria*, Senegal*, UK*
Safflower seed flour	USA
Sesame seed flour	USA*
Soy flour	Brazil*, Colombia, Japan*, UK*, USA*

Note: * Commercially processed products were available at the time in these countries. In certain instances, however, production has now been discontinued.

Other materials from pilot plants.

TABLE 5

Representative Values for Nutrients in breadmaking raw materials used in products market tested in Colombia and Senegal

Product	Moisture %	Protein %	Fat %	Ash %	Fibre %
(A) "Interpan"/Bogotá					
Wheat flour.	13.0	11.0	–	0.69	–
Rice flour.	12.5	6.7	–	0.82	–
Cassava flour.	7.1	1.46	0.2	1.3	1.72
Cassava starch.	15.9	–	–	0.16	0.15
Soy flour.	9.5	50.4	0.5	5.7	2.8
(B) "Pain de mil"/Dakar					
Wheat flour.	14.3	10.6	–	0.63	–
Millet flour. ..... (Extraction: 83 %)	9.4	11.7	4.5	1.4	–

Sources: (A) Anon. 1974. "Interpan". Joint IIT/TNO/FAO report on the Colombian-Netherlands composite flour project carried out in Bogotá 1971-72. 125p.

(B) Perten, H., Guinet, R. et Abert, P. 1972. Fabrication de pain de type français avec incorporation de farines de mils et sorghos. ITA, Senegal. AGS:SF/SEN 5 Rapport technique 8, 77p.

in comparison with imported wheat so that the profit margin of the processors is greater despite the slightly higher costs of using two or three ingredients in the place of one.

Research will have to be continued to find ways of improving technologies so that (1) higher percentages of non-wheat materials may be used in the blends without adversely affecting the acceptability of the products, (2) the use of expensive imported additives may be dispensed with, and (3) stable pre-mixes may be produced with uniform properties suited for both artisan and industrial bakeries.

Several research programmes to resolve these problems are coordinated during meetings of the Working Group on Composite Flours of the International Association for Cereal Chemistry. Discussions are held during the biennial meetings of the ICC in Vienna, and the most recent took place in May this year. Symposia are organized, in cooperation with FAO, at the site of a field project, after market tests have been successfully concluded. All countries in the region are invited to send technologists and government officials to see a pilot production line in operation, study the marketing plan and try the products for themselves. The first symposium took place in Latin America in 1972, during October in Bogota, Colombia, the next is planned for Africa in 1975 and may be held in Dakar, Senegal if adequate financial support is obtained. Other symposia for industrialists have taken place in London, organized by TPI, and in the United States, by the American Association of Cereal Chemists. The AACC established in 1973 a scientific and technical advisory group on composite flours, now called the Committe on Improved Nutrition to assist the USAID programme. Further, the International Development Research Centre in Ottawa, Canada, is sponsoring research on new mixtures at the Universities of Guelph, Manitoba and Saskatchewan, in support of a field project in Nigeria.

In view of the many different mixtures under development by national and international organizations, it has been considered advisable to prepare nutritional guidelines for the benefit of technologists, economists and government policy planners. The subject will be taken up at the next meeting of the Protein Advisory Group of the U. N. System in 1975.

TABLE 6

Biological Evaluation of Protein Quality in bread and biscuits of six Composite Flour blends

Product	Flour composition	Proportion	Protein content %	NPU	PER
Bread.	Wheat	100	10.9	37	0.82
Bread.	Wheat/rice	70/30	9.9	41	0.98
Bread.	Wheat/rice/soya	70/27/3	11.0	46	1.37
Bread.	Wheat/rice/soya	70/25/5	11.8	45	1.51
Bread.	Cassava/soya	80/20	11.3	55	2.40
Biscuits.	Cassava/soya	67/33	12.0	51	1.90
Biscuits.	Cassava/soya/caseinate	50/17/33	20.0	74	3.20
Casein (reference protein).		100			2.50

Notes: NPU = net protein utilization %. PER = protein efficiency ratio. Standardized on casein = 2.50.
Source: The use of soyflour in composite flours, de Ruiter, D. Institute for Cereals, Flour and Bread TNO, Wageningen, The Netherlands (unpublished).

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Artisanal processing and utilisation of tropical fish products

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ABSTRACT

Experimental work described in the paper seeks to demonstrate the feasibility of improving tropical fish technology at artisanal levels of operation in developing countries. Areas of recent and current research offering prospects for application in the handling and utilisation of fresh, dried, salted and lysed fish are discussed. Limitations in the transferability of work in developed countries on cold water species to tropical conditions are considered. Work is described on (1) Control of autolysis and microbial spoilage in the muscles of tropical, freshwater and marine species (mainly *Tilapias*). (2) Chemistry of South East Asian fish sauce production. Results suggest that production times may be shortened by introducing proteolytic enzymes after the initiation of lipolytic and lipoxidative actions primarily responsible for desirable flavour change. (3) Development of a salt shark cake product of good acceptability.

INTRODUCTION

The contribution of fish to world protein supplies, while not high, is increasing. It is a popular food in many developing countries, particularly those of West Africa, the Caribbean, the Pacific Islands and certain maritime South American states. Many of the newer fisheries are being developed substantially along the lines of industrialised fisheries elsewhere. In some, however, the traditional methods of handling and preservation are still in extensive use having considerable advantages over others in terms of established product acceptability. In yet others the development of traditional and imported technologies is proceeding side by side.

A considerable volume of international effort has been devoted to improving the technology of cold and temperate water fisheries industries. Some of the technology developed out of this research has been directly applicable to tropical fisheries. Much of it, however, has doubtful validity in so far as the spoilage microfloras are inherently different, the temperature-activity relationships and innate thermal stability of the tissue enzymes are different and the purchasing

powers and requirements of many tropical consumer populations are also different. For instance, the development of certain highly sophisticated technologies such as that involved in the production of "higher grade" fish protein concentrates has had a success severely limited by economic and acceptability problems in the less developed countries. Much less research has been devoted to the improvement of traditional fish preservation processes in the tropics, to an understanding of the bases for rational control of deterioration of fish in the tropics or to the transfer of valid and available technologies at appropriate levels. Illustrations are given below of research areas showing promise for application in products of well proven acceptability.

FRESH FISH. HANDLING AND PRESERVATION PARAMETERS

A body of evidence (Table 1) now indicates that, in general, tropical species of fish whether freshwater or marine can be kept under chilled conditions for considerably longer periods in an acceptable condition than cold or temperate water species. Keeping times of three to four or even more weeks are not uncommon. In recent work in our laboratory (Disney *et al* 1973; 1974) studies have been extended to a range of *Tilapia* species in African marine species. At ambient tropical temperatures (20° - 30° C) one hour at ambient temperature approximated to one day in chill (0° C) until active putrifaction approached, quality being monitored by taste panels, microbial counts and hypoxanthine concentrations as chemical indices of quality (Jones 1965).

The microbiological results are of particular interest. At ambient temperatures tilapias become inedible some 15-20 hours after death, when total viable counts are of the order or 10^3 to 10^5 organisms per gram of tissue. In iced material the groups of organisms involved in spoilage appear to be roughly similar to those in spoiling cold water species.
The methodolgy is not entirely satisfactory (Shewan, 1971; Liston, 1960; Karthiayani and Mahadeva, 1963; Velankar and Kamasastri, 1956; Watanabe, 1965). From studies on West African sea bream (Pinegar, 1974), the predominant organisms on the skin are *Pseudomonad* species but th predominant species in the flesh throughout storage are of the *Moraxella* genus (Gram negative cocco bacilli). Similar studies on *Tilapia* have found that the early predominance of micrococci and staphylococci is replaced by that of pseudomonads.

Invasion and growth of bacteria in the muscle tissues of tropical frshwater fishes is slow. In *Tilapia* species the counts of viable bacteria in the muscle tissue remain virtually zero fcr about 14 days after which they begin ro rise and reach a level of 10^5 to 10^6 bacteria after 21 to 28 days of iced storage. This shows a marked difference from iced, stored, temperate marine fishes. For instance cod attains values of some 10^8 bacteria at 14 days (Pinegar *et al.*, 1974).

It is not unreasonable to suppose that the greatly extended keeping times of tropical species in chill store relate to the susceptibilities of the spoilage microfloras and autolytic systems to the larger temperature differential applying to tropical fishes by comparison with temperate or coldwater fishes during the chilling process. Support for this thesis arises in two groups of observations that have considerable potential for application in small scale fisheries. In the first, a series of experiments conducted by the Institute's staff in Uganda, Malawi, Ghana (and in London of experimentally maintained fish) has demonstrated that gutting has little, if any, effect on the long term quality of adequately chill stored fish. Secondly, it has been

TABLE 1

Shelf life of fish stored in ice

Fish (Common Name)	Length of Acceptable Storage (days)	Reference
TROPICAL		
Marine		
Snapper (Brazil)	11-16	Watanbe (1961)
Tuna (USA)	29	Crawford and Finch (1968)
Pomfret, Mackerel, Horse Mackerel, Seer fish, Perch (India)	7-45	Velankar and Kamasastri (1956)
Sinagris japonicus (India)	27	Govindan (1971)
Bonga, Sea bream, Bumper, Burrito (West Africa)	20-26	Amu and Disney (1973)
Mixed specied (West Africa)	17-20	Aldrin *et al.* (1970)
Freshwater		
Mrigal carp (India)	35	Balakrishnan Nair *et al.* (1970)
Tilapia (East Africa)	28	Disney *et al.* (1969)
Nile perch, *Bagrus*, Lung fish (East Africa)	20-28	Hoffman *et al.* (1973)
TEMPERATURE AND COLD WATER		
Marine		
Cod	12-15	FAO, 1968 b
Haddock	12-15	
Whiting	9-12	
Hake	8-10	
Redfish	13-15	
Herring	5-6	Stroud (1972)
Mackerel	7-9	Fraser *et al.* (1968)
Freshwater		
Yellow Walleye (Canadian)	28	Dugal (1967)
Whitefish	18	Bligh (1969)
Trout (Europe)	10	Hansen (1972)

demonstrated that provided the onset of bacterial attack at tropical ambient temperatures has not proceeded too far chilling can result in a decline in the numbers of spoilage bacteria, with the effect, that in the long term, the fish keeps almost as well as that iced from the time of catching. Obviously, variables such a size of the fish and composition of the tissues affects time scales of such situations.

It may be deduced, therefore, that in many artisanal fishings from which the

traditional market requires the intact fish, modern cold or temperate water fish technologies are inappropriate. It may well no be necessary to press the use of ice upon fishermen when fishing for short periods of time. (This is not, however, to suggest that considerable improvements in canoe fisheries are impracticable, particularly in trap netting operations.) It is interesting to speculate also whether it would not be possible to obtain extended periods of storage more economically by cycling temperatures with a view to the control of spilage microfloras.

SOUTH EAST ASIAN FISH SAUCES

Fish sauces are used extensively in South East Asia as a relish for otherwise monotonous diets. They have high peptide and amino acid contents but their intake is limited by high salt concentration. There is much interest in producer countries in developing methods for the more rapid production of the sauces, if possible of lower salt content, both at industrial level and at the level of rural technology. There is interest also in the transference of the technology to other countries, for instance to West Africa. It is likely, however, that the nutritional contribution potentially available from such materials has been overestimated since it is now reasonably well established that above protein intake of about 10 % the values of protein and low molecular weight equivalents diverge rapidly.

Earlier work in our laboratory had suggested that efforts to accelerate fish sauce production through the use of proteolytic enzymes had perhaps been misdirected on the basis of a faulty understanding of the chemistry of the sauces. Certainly, it was widely accepted that the quality of accelerated sauces did not approach that of those prepared according to traditional recipes.

This suspicion is now confirmed (Dougan and Howard, 1974) from an examination of samples from China, the Philippines and Thailand and from a study of successive phases of traditional Thai production. It has been established that the characteristic sharp, cheesy odours associated with good quality are derived from the presence of formic, acetic, propionic, iso-butryic, n-buturic and iso-valeric acids to concentrations of 40-98 m. eq. per litre. Concentrations of carbonyl compounds were maximally 0.1 m-moles per litre and appeared to make little or no contribution toward flavour.

The acids were formed almost entirely during the third month of the production process. It appears that most of the fatty acids arise from the oxidation of lipid, suggesting that the approaches to fish sauce production concentrating on rapid proteolysis are unlikely to give products of traditionally acceptable flavour.

The possibility that the acids could have been formed from free amino acids by, for instance, oxidative deamination has also been considered by Dougan and Howard, who have concluded that their participation is unlikely. These observations have potential for application to the development of rapid methods of fish sauce production because they indicate that oxidation of lipids should be encouraged in the early stages. Possibly also cheap plant sources of lipase and lipoxidase may offer prospects for use.

DRIED AND SALTED PRODUCTS

The variable reports on the effects of traditional drying and salting procedures on the availability of lysine may well relate to loss of sugar contents prior to processing (Hoffman, *et al.*, 1970). Water leaching to reduce sugar content prior to

processing (Jones, 1962) is a neglected technology although it has been re-introduced recently in the context of sophisticated de-boner technique.

Variants on traditional drying and salting technology have been developed in recent years in Zambia (by Watanabe and Cabrita (1971)) and in Mexico by del Valle (1973). Further simplification for improvement of traditional techniques seems possible. For instance Anderson and Mendelsohn (1972) have described a rapid salt curing technique in which fish is covered with brine during a grinding process to limit exposure to oxygen. For such processes for the drying of minces it may be recollected that there is considerable Japanese experience on the salting of minces (Tanikawa, 1971) and also much British wartime experience of their drying (Cutting *et al.*, 1956).

The experience in our laboratory has been that satisfactory mince cakes can be formed and dried through the use of simple hand operted hamburger presses (or even in simple 'perspex' moulds at a pressure of 1,8 lbs per square inch for a period of 15 minutes). Such simple hamburger presses cost something of the order of US $70 by comparison with the $5.000 quoted by del Valle (1973) for the cost of a hydraulic press.

The salt concentrations can be varied but, in our experience, acceptability panels prefer an initial salt concentration of between 10 and 15 % on a weight basis. Such cakes are readily acceptable as del Valle has shown earlier from his Mexican experience and from those of our group in the Seychelles.

CONCLUSIONS

It is clear that potential for application of the food sciences at the levels of artisanal technology are many and that apparent simplicity in the successive stages of handling, preservation or processing can result from a fundamental approach of some sophistication.

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Use of phaseolus vulgaris in high protein-quality pasta products

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ABSTRACT

A process was developed for the manufacture of pasta products using locally produced raw materials. A mixture of 25-45-30 parts (w/w) of semolina-whole corn-peeled bean flours yielded products of high acceptability and improved protein quality. The particle size of the corn flour and the surface temperature, opening and velocity of the double-drum dryer used to effect the gelatinization of the starch in the mixture proved to be critical in the quality and acceptability of the final product. A statistically higher content of available lysine and PER values were found in the bean-containing pasta (3.8 g/16 g N and 2.02) than in a sample of commercial pasta (1.8 g/16 g N and 0.97).

INTRODUCTION

THE PASTA (spaghetti, macaroni, etc.) industry, together with the bakery industry, represent 41.2 % of the whole Central American food industry (Recinos, 1973). However, pasta products are produced basically from semolina and none of the Central American countries is a hard-wheat producer. Therefore, it would be of economical significance for the area, to establish adequate conditions for the processing of pasta products utilizing raw native materials. Furthermore, these products could be considered as a possible vehicle to improve the nutritive value of the habitual diets of these countries. This aspect is of considerable significance since pasta products are generally included in the diets of infants and children, population groups where malnutrition is more prevalent (Bressani, 1971; Viteri and Arroyave, 1973).

The possibility of ellaborating pasta products with an improved protein quality using corn and defatted soy flours as substitutes of semolina in relatively large percentages has already been demonstrated (General Foods Corporation, 1972; Molina *et al.*, 1974). The beneficial effect that a heat treatment applied to the

starchy material (corn flour) prior to the pasta production has on the quality of the final product has already been shown (Molina *et al.*, 1974).

Bressani *et al.* (1962) have also demonstrated that when the proteins of cereals and common beans (*Phaseolus vulgaris*) are combined in a 1 : 1 ratio —approximately 30 parts of beans and 70 parts of cereal— maximum protein quality of the mixture is obtained. Since soybeans are not currently produced in the Central American area where beans are a common staple, it was decided to examine the possibility of producing high protein-quality pasta products using a semolina-corn-bean mixture, maintaining the proportion of beans at 30 % (w/w).

The present work describes the process developed to prepare pasta products of high acceptability and high protein-quality from a mixture of semolina-corn-bean flours in the proportion of 25-45-30 parts (w/w).

MATERIALS AND METHODS

THE COMMON CORN (*Zea mays*) used in this study was an open-pollinated variety ("Azotea") from the 1973 crop, grown at INCAP's experimental farm, "San Antonio Pachalí", Guatemala, at an altitude of 1,480 m above sea level. The semolina and the common beans (*Phaseolus vulgaris*) were obtained locally.

The beans were hand-peeled, and then ground in a hammer mill equipped with a 40-mesh screen. Whole corn was ground in the same mill.

The whole corn flour, peeled bean flour and semolina were mixed in the proportions specified later in the text using a Patterson-Kelley blender (Model LV-16 qt). An equal weight of tap water was added stepwise to the mixture prepared, in order to obtain a dough. Such dough was hand-fed to a double-drum dryer (General Food Package Equipment Corporation, GF series, Model 215) which was operated under the conditions detailed later on. The pasta products (spaghetti type) were then prepared from the mixtures thus treated, using a Euro-Milan (Model TR-5) pasta-making machine. For this purpose, lots of 3 kg of each flour mixture were added to the mixing bowl of the pasta-processing machine. Enough tap water —between 240 and 270 ml per kilo of flour— was added stepwise to the flour mixture with constant agitation to obtain the right consistency of the dough. The total mixing time was between 35 to 45 min. After this operation the trap-door connecting the mixing bowl with the single screw extruder of the pasta-making machine was opened and the dough passed through the extruder at the single speed available in the machine. All pasta products were air-dried at 40° C to 45° C in a locally built tray-dryer (similar to the Schilde Simplicitor dryer, Model SG 5/XII) for 12 to 14 hours. The relative humidity of the drying air at such temperatures oscillated between 17 and 20 %. The average diameter of the resulting sphaghetti was 2.44 mm.

Nitrogen, ash, ether extract, moisture, crude fiber and starch were determined in duplicate according to the AOAC method (1970). Protein was estimated multiplying the nitrogen content by 5.70 in the case of semolina, and by 6.25 in the case of the whole corn and peeled bean flours. Similarly, in the case of the commercial pasta the conversion factor of 5.70 was used, while for the pasta prepared from the semolina-corn-bean (25-45-30) mixture studied, a calculated 6.12 conversion factor was adopted to compensate for the proportion of semolina.

Total sugars were determined using the method described by Pomenta and Burns (1971). The content of available lysine was determined following the method described by Conkerton and Frampton (1959). Damaged starch was established according to Farrand (1964).

Using the methods of the AACC (1969), tests were carried out in all pasta products to determine water absorption during cooking, volume increase as the result of cooking, and resistance to disintegration. In the latter test, a standard cooking time of 20 min was adopted. The organoleptic evaluation of the pasta products was carried out using the consumer preference tests described by Kramer and Twigg (1966), and a panel of 10 semi-trained individuals. Numerical values of 9, 7, 5, 3 and 1 were assigned to the likeness levels of the hedonic scale. Prior to testing, all samples were home-cooked under equal conditions.

The protein efficiency ratio (PER) was determined on the uncooked pasta product, essentially by

the AOAC method (1970). Weanling rats of the Wistar strain from the INCAP animal colony were distributed in groups of 3 males and 3 females each. All diets were supplemented with a 4 % salt mixture (Hegsted *et al.*, 1941), 5 % cottonseed oil, 1 % cod liver oil and enough corn starch to adjust to 100 g to which 5 ml of a vitamin B solution (Manna and Hauge, 1953) was added.

RESULTS AND DISCUSSION

The chemical composition (on "as is" basis) and available lysine content of the semolina, whole corn and peeled bean flours are presented in Table 1. As was to be expected, a higher protein content was found for the peeled bean flour than for the semolina and whole corn flour. Conversely, the latter materials presented a higher starch content than the former. From a nutritional point of view it is of interest to note the much higher available lysine content presented by the peeled bean flour when compared to that determined in semolina or the whole corn flour. The relatively high protein and available lysine content of the peeled bean flour suggest the possible use of this material as a protein supplement for pasta products prepared from a mixture of semolina and corn flour.

TABLE 1

Percent composition and available lysine content of semolina, and whole corn and peeled bean flours

Component	Semolina	Whole corn flour	Peeled bean flour
Moisture	13.22	13.11	15.46
Protein	14.72	9.21	23.10
Ether extract	1.41	4.27	1.92
Crude fiber	0.32	1.75	0.48
Ash	0.71	1.83	3.72
Starch	68.21	69.31	39.51
Total sugars*	1.80	1.38	3.35
Available lysine (g/16 g N)	2.48	3.05	7.82

* Expressed as glucose.

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Since Mayorga (1973) and Molina *et al.* (1974) have shown that a heat treatment of the corn flour is necessary to obtain pasta products of an acceptable quality when using a 32-60-8 semolina-whole corn-defatted soy flour mixture, the possibility of using a double-drum dryer to apply such heat treatment with a 25-45-30 semolina-whole corn-peeled bean flour mixture as a basic formula was studied. The degree of gelatinization obtained through the heat treatments was evaluated by measuring the damaged starch content of the processed samples (Farrand, 1964; Mayorga, 1973).

The relationship between the damaged starch content of the semolina-whole corn-peeled bean flour (25-45-30) mixture subjected to 4 different thermal treatments and the solids in cooking water value obtained for the pasta products prepared from each mixture are shown in Figure 1. The 4 drums' surface temperatures evaluated were 85°, 96°, 130° and 143° C which corresponded to a drum internal steam pressure of 0.70, 1.05, 2.11 and 4.22 kg/cm^2 (10, 15, 30 and 60 psig), respectively. The drums' velocity was 5 rpm and the opening between them

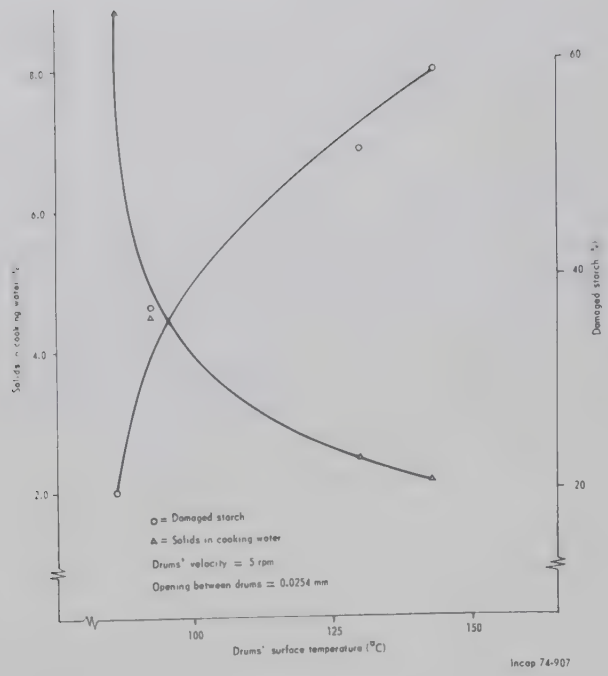


Fig. 1.–Effect of drums' surface temperature on the damaged starch content of the 25-45-30 semolina-whole corn-peeled bean flour mixture, and on the solids in cooking water value from the corresponding pasta products.

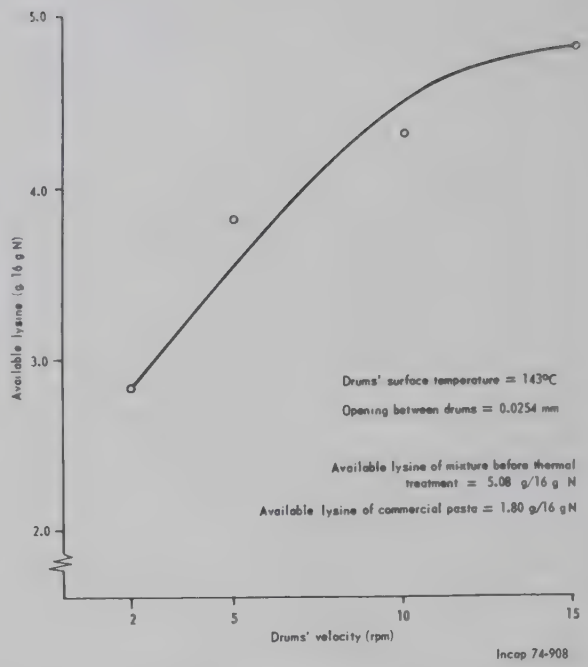


Fig. 2.–Effect of drums' velocity on the available lysine content of pasta prepared from a semolina-whole corn-peeled bean flour mixture (25-45-30).

was 0.0254 mm (0.01 in) to insure a good starch gelatinization (Anderson *et al.*, 1969).

As the Figure reveals, there is an inverse relationship between the damaged starch content of the mixtures and the solids in cooking water value obtained for the pasta products prepared therefrom. The correlation coefficient found for both parameters was -0.91.

Based on the above findings the drums' surface temperature was fixed at 143° C for the following experiments. On the other hand, since varying the drums' velocity from 5 to 15 rpm using the above-mentiones surface temperature (143° C) and drum opening (0.0254 mm) had relatively little effect on the damaged starch content of the mixture, we considered it appropriate to study the effect that the drums' velocity could have on both the protein quality and the acceptability of the final product.

The effect of the drums' velocity on the available lysine content of the pasta product is presented in Figure 2, where the available lysine content of a commercial pasta and of the mixture prior to the heat treatment in the drum dryer, is also shown. As can be observed, decreasing the drums' velocity had a detrimental effect on the available lysine content of the final product. However, in all cases the available lysine content of the pasta product prepared from the mixture studied was higher than that determined in a commercial pasta obtained locally.

Figure 3 illustrates the effect of the drums' velocity on the PER values of the pasta products.

The decrease of the drums' velocity had a detrimental effect on the PER value of the final product, similar to that observed on the available lysine content (Fig. 2). In fact, a correlation coefficient of 0.94 was found between the available lysine content of the samples (Fig. 2) and their corresponding PER values (Fig. 3). In view ot this correlation, a biological trial was carried out with a pasta prepared from a 25-35-40 semolina-whole corn-peeled bean flour mixture processed at a drums'

velocity of 5 rpm; the trial revealed a higher available lysine content (4.7 g/16 g N) than the pasta prepared with the 25-45-30 mixture (3.8 g/16 g N) processed under similar conditions (see Fig. 2). Although the PER value obtained for the former product (1.97) was similar to that of the latter one (2.02), the idea of increasing the beans proportion in the mixture was considered of interest since it would increase the total protein content of the final product.

The organoleptic score and the solids in the cooking water value determined for the pasta products containing 3 different levels of peeled bean flour in the mixture with a constant 25 % of semolina and processed at 4 drum velocities are given in Table 2. As may be observed, at any given drum velocity studied, an increase in the beans proportion in the mixture had a detrimental effect both on the organoleptic score and on the solids in the cooking water value obtained in the final product. An increase in the drums' velocity, at any bean flour level in the mixture, also had a detrimental effect on both parameters. Analysis of the data using Duncan's test (Duncan, 1955) revealed that according to the values attained for both parameters, the pasta products statistically equal ($P < 0.05$) to the commercial pasta used as standard, were those prepared from a mixture containing 30 or 35 % bean flour when processed at 2 rpm, and from the mixture containing 30 % bean flour processed at 5 rpm. From the above-mentioned results and from the protein quality findings presented earlier (Figs. 2 and 3) use of the 25-45-30 semolina-whole corn-peeled bean flour mixture processed in the drum dryer at a surface temperature of 143° C, with an opening of 0.0254 mm and a velocity of 5 rpm was agreed upon for any further studies. Increasing the proportion of corn to 55 at the expense of semolina resulted in an organoleptic score of the product (4.3) statistically lower ($P < 0.01$) than that of the standard pasta (7.00).

TABLE 2

*Effect of drums' velocity and percentage of peeled bean flour in the mixture on the organoleptic score and the solids in cooking water value of the pasta products**

	Drums' velocity (rpm)**							
	2		5		10		15	
Mixture***	Organo-leptic score	Solids in cooking water (%)	Organo-leptic score	Solids in cooking water (%)	Organo-leptic score	Solids in cooking water (%)	Organo-leptic score	Solids in cooking water (%)
25-45-30. .	7.63	2.24	7.00	2.28	4.26	2.36	–	3.13
25-40-35. .	7.53	2.27	6.05	2.39	2.43	2.53	–	3.26
25-35-40. .	7.31	3.02	5.60	3.59	2.43	3.79	–	5.37

* The standard values obtained for commercial pasta were: organoleptic score = 7.00 and solids in cooking water = 2.01 %.

** Drum's surface temperature = 143° C. Opening between drums = 0.0254 mm.

*** Semolina-whole corn-peeled bean.

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The volume increase and water adsorption during cooking as well as solids in cooking water and protein content determined for the pasta product obtained from the 25-45-30 mixture processed under the above-mentioned conditions, are detailed in Table 3. Values for the commercial pasta used as standard are also included. It is

TABLE 3

Quality characteristics of commercial pasta and the pasta product prepared from the 25-45-30 semolina-whole corn-peeled beans flour mixture

Product evaluated	Volume increase during cooking (%)	Water absorption during cooking (%)	Solids in cooking water (%)	Protein (%)
Pasta from the semolina-corn-bean mixture	80	373	2.28	15.61
Commercial pasta.	151	197	2.01	14.71

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interesting to observe that while the water adsorption during cooking is significantly ($P < 0.01$) greater for the semolina-whole corn-peeled bean product, the standard commercial pasta presents a significantly ($P < 0.01$) higher volume increase during cooking. The solids in cooking water and the protein content, on the other hand, were very similar in both cases.

Evaluation of the effect that the opening between the drums used for processing the mixture could have on the quality of the final product demonstrated that essentially the same results reported in previous paragraphs using an opening of 0.0254 mm were obtained with larger openings up to 0.2032 mm (0.008 in). It was also found that in order to improve the appearance of the uncooked pasta product, eliminating the spots of gelatinized corn starch granule which appeared on the surface of the product, a whole corn flour of 100 mesh or finer had to be used.

The flow diagram of the proposed process and the balance of materials obtained at pilot plant scale presented in Figure 4, was prepared based on the

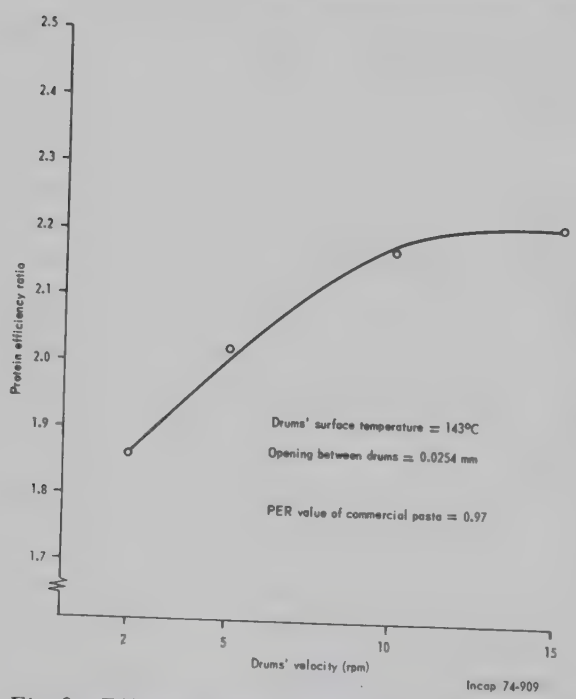


Fig. 3.–Effect of drums' velocity on the protein efficiency ratio (PER) of pasta prepared from a semolina-whole corn-peeled bean flour mixture (25-45-30).

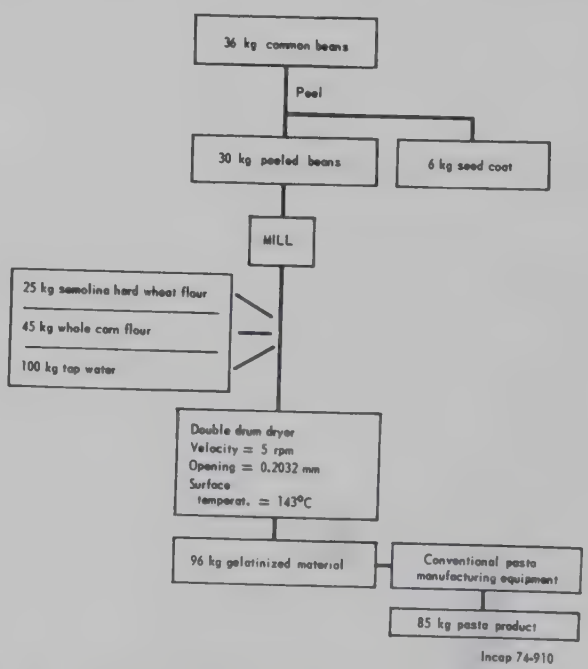


Fig. 4.–Flow diagram and balance of materials for the manufacture of pasta products from a 25-45-30 semolina-whole corn-peeled bean flour mixture.

afore-mentioned findings. The peeling operation is considered necessary since when a whole bean flour was used, the seed coat always separated from the final product during the cooking operation and tended to float in the cooking water, thus impairing the quality of the product. This effect was observed even when a 200-mesh whole bean flour was used. Although the peeling operation in the present study was done by hand, the equipment for such operation is already commercially available (Kon *et al.*, 1973).

Cooking the peeled bean flour (15 min at 15 psig or 121° C) prior to the processing of the mixture had no effect in improving the nutritive value of the final pasta product. This indicates, therefore, that the thermal treatment given to the mixture through the drum dryer was enough to inactivate the growth-inhibiting substances contained in the common bean portion (Liener, 1962).

Preliminary data indicate that the corn variety used in this study ("Azotea") can be substituted by other corn varieties, such as opaque-2 corn or precooked ("nixtamalizadas") corn flours, commercially available, without any detrimental effect on the quality of the final product. The peeled common bean flour can be substituted as well by a peeled black-eyed pea (*Vigna sinensis*) or a peeled chick-pea (*Cajanus cajan*) flour with similar results.

At present we are investigating the possibility of effecting the desired thermal treatment of the mixture by means of other equipment than the drum dryer.

Preliminary cost analysis of the proposed process has proven satisfactory, mainly due to the difference in price between the semolina and the corn and beans. The versatility of the equipment used to effect the thermal treatment also helps to make the present process economically and industrially appealing.

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Vegetable proteins for combating protein malnutrition in developing countries

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ABSTRACT

In India and other developing countries the State Governments have launched programmes of nutritional intervention in the case of vulnerable groups of population. Since milk is in short supply, necessarily these programmes are dependent on oil seed protein concentrates. In India edible peanut flour is used in such programmes. The foods based on this which are now used, are Bal Ahar and Miltone. Bal Ahar is a dry blend of flours from cereals, oil seed meals and pulses. Several such compositions are used in these programmes. Miltone which is a milk like preparation contai: ing peanut protein isolate, is being produced at the Bangalore Dairy for the Special Nutrition Programme for children below 6 years. The paper describes, the chemical composition and nutritive value of these foods. Details of the programme and the use of Miltone are described.

INTRODUCTION

The magnitude of malnutrition amongst the population in the developing countries has been conservatively estimated to be about 50 % (Sukhatme 1967). The preschool and school children are the major sufferers. Malnourishment is linked with the economic status of population. The calorie intake as well as the intake of proteins and vitamins tend to go down with the income of the family. Recent published data in India has shown that between 20 to 40 % of the people do not have a *per capita* consumption level of more than Rs 40/-($5/-) per month and hence have been classified as below the "poverty line." (Anonymous. 1973) In India the animal protein is only 10 % of the total protein in the diet (55 g). The consumption of animal protein (milk, meat and oggs) is also linked with the income. (Anonymous. 1972).

Several reasons have been put forward for the cause of malnutrition in the developing countries. But overall it is the problem of over population and under

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production. The reasons for under production in a country which is predominently agricultural like India, have been discussed by Swaminathan (1973). These are, the low yields of crops as compared even to the minimum yields of similar crops elsewhere (2) the large percentage of population involved in the production of food (72 %) and (3) the dependence of agriculture on monsoon which is highly unpredictable. The nutritional content of the meals and the overall incidence of malnutrition is a consequence of this agricultural situation. The calorie and protein content of the average Indian meal in 1940 was 1950 and 52.2 g. and in 1969 it was 1940 and 48 g. In the same period the calorie and the protein contents of the meals in Japan showed an increase from 2020 and 60 g. to 2450 and 75 g. This improvement in the nutrition value of the Japanese diet has resulted in marked improvement of the health of the younger generation.

The effect of malnutrition of the young population (42 % of the total Indian population is below 14 years) on the economics and future welfare of the state has attracted considerable attention in Indian and in other developing countries. (Muscat and Berg 1971). The task Force on Nutrition of The National Committee for Science and Technology, appointed by the Government of India has brought this out in its recommendations and has emphasised the need for an overall effort to remedy this. The cost of malnutrition of the growing population to the state is made up of several components like (a) providing sustenance and maintenance to a large number of malnourished and possibly mentally retarded individuals and the effect of this on the well-being of the state (b) the cost of treatment, hospitalization and rehabilitation of the malnourished and (c) overall loss of the investment in the death of the malnourished. In countries like India where a large section of the juvinile population is under or mal-nourished, the cost of such avoidable losses could be enermous. Realising this and with the object of overcoming the national waste, the Government of India has launched massive welfare programmes for nutritional intervention of the vulnerable groups.

NUTRITIONAL PROGRAMMES:

These programmes could broadly be divided into (a) programmes which are concerned with the feeding of the school going child (b) programmes which are specially concerned with the feeding of the preschool children, infants, the lactating mothers and pregnant women (c) programmes which are directed towards the amelioration of mal-nourishment amongst the children of the tribal people and (d) programmes which are composite in nature and which involve education or family planning approach along with nutrition. Most of these programmes are based on provision of food which is locally available. In India, the production of adequate milk has many constraints. Though India happens to be third in the production of milk in the world, still the amount of *per capita* availability of milk is extremely small (110 ml.). Government projects, which are trying to augment the milk production are multipronged in approach. They aim at (1) better utilization of land to produce cattle fodder (2) improvement of the breed by cross fertilization and artificial insemination (3) better provision of roads and other facilities to the villages, which are the main milk producing centres and (4) better utilization of the milk by proper collection, processing and distribution. In spite of the great emphasis that has been placed on these measures, the overall production of milk is still laging and cannot catch up with the increasing population. Milk is available only in limited quantities and at a price which often is beyond the purchasing

capacity of a large section of the population. Imported milk powder was being used in the nutritional programmes but the dwindling stocks of milk powder in the producing countries and the increase in cost have made this unavailable now. Use of locally produced milk or milk powder in these nutritional intervention programmes is thus not possible.

EDIBLE OIL SEED FLOURS FOR FOOD FOR THE VULNERABLE GROUPS:

An alternative source to milk protein in India is protein from edible oil seed meals. Oil seed meals are comparatively cheap, as they are the by-products of the oil industry. A kilo of protein from milk costs about $7/-. While from imported skim milk powder it costs $5/-. But protein from peanut flour costs only $0.53/-. Thus as a source of cheap protein, edible oil-seed-meals are very attractive. Besides use of edible oil seed meal as human food, is a shorter food chain than use of it as food for cattle, for production of protein in milk or meat. The available sources of oil seed meals in India are shown in Table 1. Standards have been laid down for edible oil seed flours from peanut (De. 1971, ISI, 1968) cotton seed and soya (PAG. 1965, ISI. 1969). The method for the production of the flour from peanut has been standardized by the Central Food Technological Research Institute, (Subrahmanyan et. al., 1957 a.) The peanut flour produced according to the above method is free from the red cuticle and the germ. A process for the production of edible sesame flour has also been standardized by Shamanthaka Sastri, et al., (1969) and standards for it prescribed. (PAG. 1973). The most abundant sources of vegetable protein are peanut flour and cotton seed flour. The cultivation of soya was started on a large scale only recently, and the quantity available for use as food suppliments is limited.

TABLE 1

Production of Major Oil seeds & Legumes in India and their Potential Protein Supply During 1971

(FAO Production Year Book 1972)

Oil seeds and legumes	Area x 1,000 ha	Average yield 100 Kg/ha	Production x 1,000 MT	Protein equivalent x 1,000 MT
Cotton*.	7,610	2.2	1,641	328
Groundnut Kernels. ..	6,900	6.3	4,250	1,088
Sesame.	2,500	1.8	460	92
Rapeseed.	3,321	5.9	196	36
Copra.	—	—	280	21
Chick peas.	7,810	6.7	5.247	1.049
Pigeon peas.	2,648	7.0	1.854	371
			Total	2,985

* The data is for 1970.

The problem in the utilization of oil seed flours in human foods are (a) production of the flours in a sanitary and hygienic way (b) elimination of toxic constituents present in them and (c) enhancing the low nutritional value of the flours.

The antinutritional and toxic factors in the oil seed meals are well recognized and characterised. (Liener 1969) Methods for removal or for bringing them below toxic levels have been published. In peanut meal, the level of the adventitious toxin, aflatoxin, can be controlled in several ways. According to the Mysore method (Subrahmanayan et. al. 1957 a) the toxin is controlled by carefully picking out the fungus affected seeds twice before expelling for oil. Methods for complete removal of the toxin by chemical methods have also benn published. (Srinivasamurthy et. al. 1967 and Gardner et. al. 1971) In India peanut protein concentrate is mostly used in child feeding programmes. Edible cotton seed meal is not used because it is relatively costly. There are at present no large scale units for production of gossypol-free cotton-seed flour in the country. Moreover the colour of cooked cotton seed flour is not acceptable. Soya flour has been recently introduced and as yet large quantities are not available for these programmes. However, the CARE organisation is importing soya flour and using it in its programmes. Sesame flour is still in the development stage.

The amino acid composition of the edible flours in shown in Table-2. By themselves the oil seed meals are low in nutritive value (PER) but when supplemented with each other or with a good protein like milk, their PER can be raised to that of casein or even of milk. This principle is used in the formulation of the foods for the Welfare programmes.

TABLE 2

Amino Acid Composition of the Proteins of Principal Oil Seed Meals, Legumes and Milk

Sl. No.	Name of Material	% Protein in seed or milk	Amino acid composition (g per 100 g protein)							
			Iso leucine	Leucine	Lysine	Total S. Amino acids	Phenyl alanine	Threo nine	Trypto phan	Valine
1	Groundnut	25.6	3.38	6.40	3.53	2.40	4.98	2.61	1.04	4.18
2	Soya been	38.0	4.54	7.78	6.38	2.59	4.94	3.86	1.28	4.80
3	Sunflower Seed	12.6	4.27	6.42	3.60	3.39	4.45	3.68	1.36	5.07
4	Sesame	18.1	3.61	6.70	2.74	4.62	4.43	3.57	–	4.61
5	Cotton Seed	20.2	3.30	5.92	4.42	2.85	5.22	3.30	1.25	4.64
6	Niger Seed	17.3	4.21	6.56	4.43	3.18	4.26	3.87	–	3.63
7	Bengal gram	20.1	4.43	7.49	6.85	2.22	5.73	3.76	–	4.54
8	Milk (Cow)	3.5	4.72	9.53	7.79	3.33	5.38	4.45	–	5.79

FOODS USED IN NUTRITIONAL INTERVENTION PROGRAMMES:

The new foods that are being used in the nutritional intervention programmes in India can be classed into three catagories: (1) high protein foods (2) medium protein foods and (3) milk like preparations. A high protein food under the name of India Multipurpose Food was used in school feeding programmes as a supplement to the staple food (Subrahmanyan et. al., 1957a). This was a blend of 75 parts of edible peanut flour and 25 parts of chick peaflour (cicer arietinum). It was fortified with vitamin A, vitamin D, thiamine, riboflavin and nicotonic acid and with calcium phosphate. Studies have shown its beneficial effect in post-Kwashiorkar treatment as well as in preventive treatment of malnutrition, (Subrahmanyan et. al., 1957 b) Though the benefits of its use in these cases, has been well established and

its price is comparatively low (about $0.50 per Kg) it is now used only in a restricted quantity and only one unit is producing this. The reasons for its limited success are (a) it was not backed by marketing strategy and (b) private sector units were reluctant to produce it, as the profit margin was narrow. (Orr. 1972).

Bal Ahar: The most widely used food at present in the programmes is "Bal Ahar". This name is a generic term meaning children's food. Under this caption, several formula have been tested and used. The one that was originally in production was a blend of seventy parts of whole wheat flour (**atta**), fifteen parts chick pea flour and fifteen parts peanut flour. On considerations of cost the chick pea flour was substituted by soya flour. The food is fortified with vitamins and calcium phosphate. The chemical composition and amino acid composition of the blends are shown in Table. 3. The CARE organisation in India is supplying CSM which is a blend of 85 parts of bulgar wheat flour and 15 parts of solvent extracted soya). Bal Ahar is being produced by several flour millers in the country for the government. During 1972-73, 25,000 tonnes of Bal Ahar were produced and it is projected that during 1974, the production will be 40,000 tonnes. Recently attempts are being made to replace the imported content in Bal Ahar (the bulgar wheat and soya), by indigenously available raw materials. A blend under the name of Energy Food has been standardised and is under trial in a block at Chitaldroog (Karnataka State) where nutrition is being combined with family planning. A similar attempt is

TABLE 3

Composition of Foods used in the Nutritious Programmes in India

Constituents	Bal Ahar Formula I	Formula II	Formula III	Formula IV	Formula V	Energy Food		Win Food	KIF
Wheat.	–	70	–	70	–	15		–	–
Bulgar Wheat (soy fortified). .	85	–	85	–	95	–		–	50
Bajra*.	–	–	–	–	–	–		40	–
Ragi**.	–	–	–	–	–	22		–	–
Tapioca Fluor. . . .	–	–	–	–	–	–		–	25
Edible Peanut Flour.	15	30	–	15	–	15		25	25
Soy flour (defatted).	–	–	–	15	–	–		–	–
Chick pea flour****.	–	–	15	–	5	15	Greengram*** flour	15	–
						23		20	–
				Brown sugar.					
				Hydrogenated fat. .		5		–	–
				Salts.		5		–	–
Protein content. . .	22.0	23.0	18.0	22	18	17		20	21.0
Energy content (Kilo calories). .	359	357	357	359	355	432		332	359
Amino Acids g/16 g Nitrogen calculated									
Lysine.	4.0	3.3	4.6	4.0	4.4	4.0		4.0	3.8
Total S. amino acids. . .	3.0	3.0	3.1	3.2	3.2	2.9		2.7	2.8
Threonine.	3.2	2.7	3.6	3.2	3.5	3.0		3.0	3.0

**Pennisetum typhoideum. **Eleusine Coracana. ***Phaseolus Aureus. ****Cicer arietinum.*

the production of Win Food in Tamil Nadu and KIF in Kerala. The composition of these are shown in Table 3.

Extruded foods are also being tried. An experiment to assess the acceptability of extruded Bal Ahar with soya bean oil sprayed on it is being tested by the Amul organization (the foremost baby food manufacturers in India) in a village near Surat (Gujarat State). Similarly the CARE organization has set up a unit in Andhra Pradesh State for the production of ready-to-eat foods in the form of fried macaroni.

Miltone:

Milk like preparations based on oil seed meals were first prepared by Subrahmanyan et. al. (Anonymous. 1955) In these early preparations, either peanuts or soya were used as such. The resulting milk always had a characteristic smell of soya or peanut from which it was made. Hence marketing of these was difficult. In the modified process of preparation of Miltone, (Chandrasekhara et al., 1971) the protein is isolated from solvent extracted edible peanut flour. To enhance the nutritive value and its organoleptic acceptability, it is mixed with an equal quantity of milk. The flow sheet of the process is shown in Fig. 1 and the composition in Tables 4 and 5. Miltone has nearly the same physical and chemical characteristics of milk. The protein is a blend of equal parts of peanut protein and milk protein, 50 % of the carbohydrate is sucrose. The fat which is entirely from milk is only 50 % of what was in the milk used. Feeding trials have been conducted on Miltone and spray dried preparations resembling Miltone (Chandrasekhara et al., 1972). These are summarized in Table. 6 & 7. The feeding trials have shown that Miltone compares well with milk in providing nutrition to the growing children. Miltone has been in production for the past seven years at the Bangalore Dairy. From a production of about 400 litres of Miltone per day the production has gone

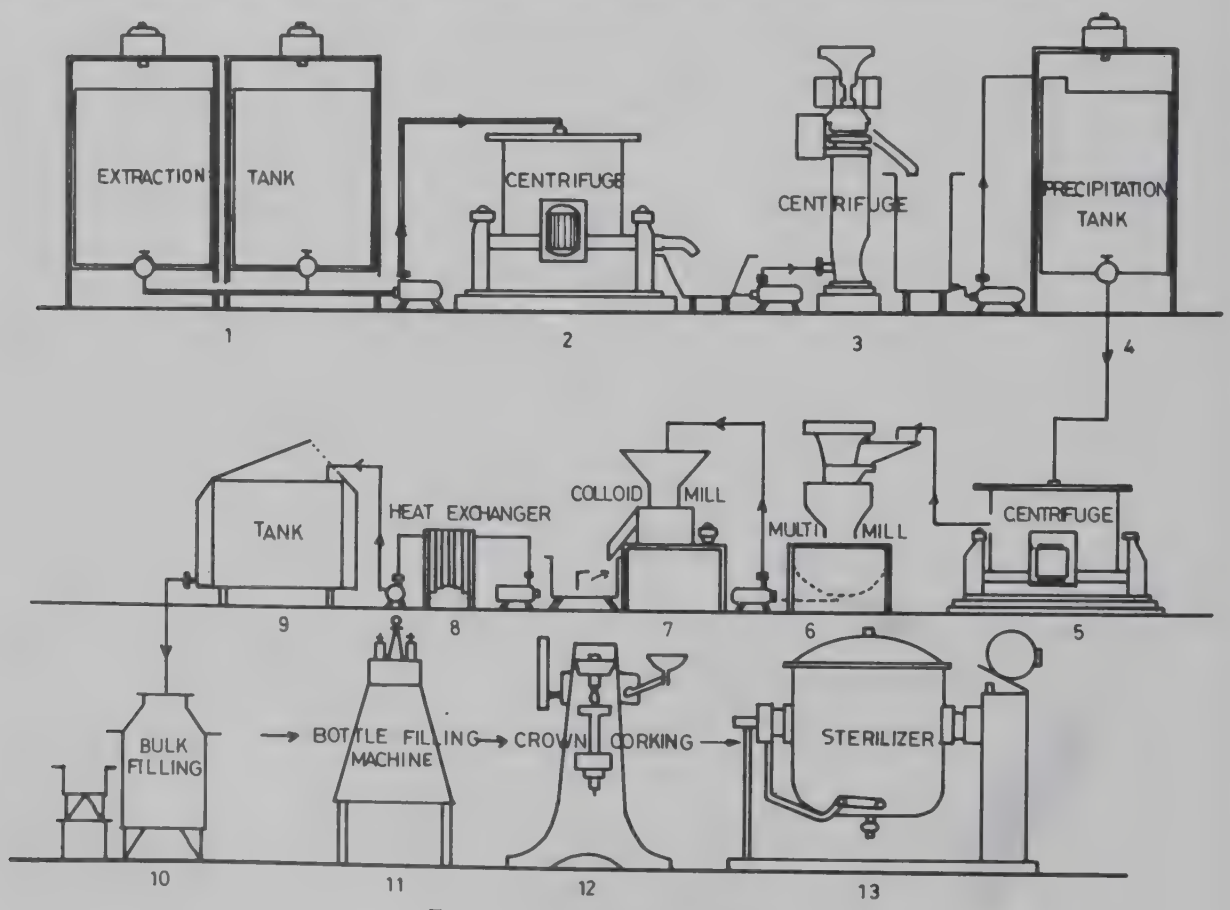


Fig. 1.–Flow sheet for miltone.

TABLE 4

Chemical Composition of Miltone

Constituents	Values per 100 g
Total Solids.	11.5 g
Fat.	2.0 g
Protein.	4.0 g
Carbohydrate.	5.0 g
Vitamin A.	150 IU
Vitamin C.	6.0 mg
Vitamin D.	16 IU
Vitamin E.	0.8 mg
Thiamine.	0.04 mg
Riboflavin.	0.15 mg
Pyridoxine.	0.025 mg
Niacinamide.	0.10 mg
Calcium Pantothenate.	0.35 mg
Vitamin B_{12}.	0.27 g
Folic acid.	0.2 g

TABLE 5

Amino Acid Composition of Miltone and Miltone Curds (Yoghurt)

Amino Acid	g per 16 g Nitrogen			
	Miltone	Miltone Curd	FAO Pattern	Egg+ Protein
Lysine.	4.2	4.4	4.2	7.0
Methionine.	1.9	2.1	2.2	3.4
Total S. amino acids.	3.3	3.3	4.2	5.8
Phenylallanine.	5.4	4.9	2.8	5.7
Leucine.	4.2	4.5	4.8	8.8
Iso-leucine.	4.8	4.8	4.2	6.2
Valine.	6.1	6.0	4.2	6.8
Arginine.	10.2	9.9	–	–
Threonine.	3.6	3.4	2.8	5.1
Tryptophan.	1.3	1.2	1.4	–
Available lysine.	2.6	4.2	–	–
Chemical (Egg protein) score.	57 (TSA)*	57 (TSA)*		–
Chemical (FAO pattern).	78 (TSA)*	78 (TSA)*		

* On the basis of Total Sulphur Amino Acids.
+ Amino Acid Content of Foods. 1970 FAO. Rome.

upton 6,000 litres per day. (Fig. 2). The entire quantity is now being used in the Special Nutrition Programmes in urban slum area of Bangalore.

Miltone is well adopted to this programme because of the following reasons: (a) it doubles the quantity of milk available for these programmes without using scantily available imported skim milk powder (b) the cost of Miltone is much less than of milk, (the selling price at Bangalore of one litre of milk with SNF 9.8 and Fat

3.8 is $0.21 and of Miltone with SNF 9.0 and Fat 1.9 is $0.14), (c) it has a high acceptability with children (d) it is well suited for providing nutrition to the infants who cannot take only bread. (Fig. 2) However, since only Miltone cannot provide the the calories required, it is given along with bread. The ration is shown in Table 8.

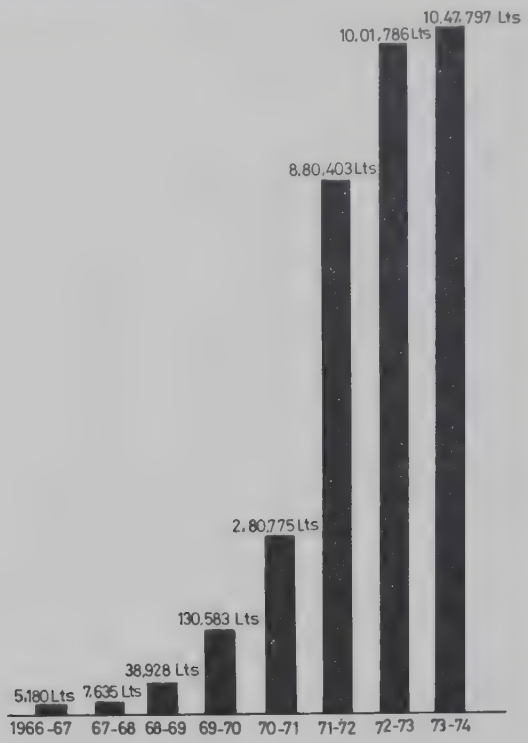


Fig. 2.–Annual production of miltone.

TABLE 6

Protein Efficiency Ratio of Miltone, Sterilized Miltone and Miltone Curds

Treatment	Average initial weight	Average gain in weight	Average protein consumed	Average PER	Standard PER
A. Miltone as such.	32.5	22.0	60.1	2.7	2.5±.046*
B. Miltone sterilized.	32.5	19.9	51.8	2.6	2.3
C. Miltone lactic fermented (curd)..	32.6	23.9	72.0	3.0	2.7±.05*
D. Control Milk.	32.7	28.3	93.8	3.3	3.0±.046*

*** Standard error on 27 degrees of freedon.**
**** Average based on 7 observations only.**
Test of significance A B 0.14(5 %); B C 0.43(0.1 %).
All three treatments significantly (0.001) lower than control.

The success of utilization of Miltone in this programme at Bangalore has encouraged the government to set up two more such units (production capacity 5000 litres per day) at Hyderabad and at Ernakulam. These units will go into production during September of this year. It is proposed to set up four more such units during the current year.

A large unit with a capacity of 24,000 litres of Miltone per day is being set up at the Bengalore Dairy. This unit will require automatic centrifuges and decanters which are not being made in India at present. UNICEF has been a partner in these projects since the time they were concieved and is now purchasing these

TABLE 7

Infant feeding trial with Miltone and Control Toned Milk

Group	Supplement given	No. of infants	Weight Kg: Average initial	Weight Kg: Average final	Weight Kg: Average increase ±SE	Height cm: Average initial	Height cm: Average final	Height cm: Average increase ±SE
A.	Miltone.	22	6.0	7.2	1.2±0.1	65.8	70.2	4.4±0.1
B.	Toned Milk. . .	19	5.8	7.2	1.4±0.1	64.6	69.2	4.7±0.1
Difference and results of significance					0.2 N.S.			0.3 N.S.

N.S. = Not significant (P 0.05).
Results of test of significance based on two tailed test and standard errors are calculated from the pooled variance based on 39 degrees of freedom.

TABLE 8

Special Nutrition Programme
Supplementary Feeding Regimen for different Age groups

Age (months)	*Food Supplements*
0-6.	225 g Miltone
7-12.	225 g Miltone and one slice (35 g) bread
13-36.	225 g Miltone and three slices (100 g) bread
37-72.	84 g Miltone and two slices (66 g) bread
Pregnent women (Last three months) Lactating Mothers (first quarter)	84 g Miltone and 3 slices (100 g) bread.

non-indigenous equipment for the plant. It is expected that this plant will be in operation by the end of 1975.

Miltone can also be sterilized to provide a beverage. For this, Miltone is generally flavoured and used. This is filled in 200 ml. capacity bottles which are crown corked and sterilized at 15 psi for about 15 minutes in a revolving steam sterilizer. The shelf-life of this product is more than six months even at ambient temperatures. It has found excellent acceptance among school children. One bottle of sterilized Miltone provides 8 g. protein and about 110 kilo calories besides vitamins and minerals.

In the nutritional intervention programmes of the Indian Government to improve the health of the preschool and shool children, oil seed meals offer an excellent medium. Processed products based on these like Bal Ahar and Miltone provide acceptable foods for such programmes.

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Preparation and evaluation of dehydrated textured protein product from coconut protein

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ABSTRACT

A simple and inexpensive process for producing dehydrated textured protein cubes and granules from coconut protein concentrate is described. Protein concentrate, prepared by heat-coagulation of coconut skim milk was combined with mung bean (Phaseolus aureus, Roxb.) flour, food yeast, vegetable oil, binder, emulsifier, flavor and color. The mixture was precooked and dried in the form of cubes or granules. The dehydrated product contained on the average 52 per cent protein (D.W. basis) and fairly high values for calcium, phosphorus, iron and thiamine. Methionine plus cystine were the major limiting amino acids. Sensory evaluation of recipes prepared from the two types of products showed high acceptability during the six months storage period. Results indicate that coconut protein concentrate can be used for the manufacture of meat product substitutes.

INTRODUCTION

The use of edible vegetable protein to improve the nutritional value of many familiar foods or as a source of new food products either to replace and/or extend the more expensive animal proteins is a major advancement in the food field. According to the USDA (1971) "textured vegetable protein products are food products made from edible protein sources and are characterized by having structural integrity and indentifiable texture such that each unit will withstand hydration in cooking and other procedures used in preparing food for consumption". Plant protein products, with or without meat-like structural characteristics are now produced on a large scale principally from soybean protein and are available in various shapes, sizes and flavors (Robinson, 1972).

Coconut protein is a substantial source of potentially available food protein in many tropical countries. The Philippines is the largest single producer of coconut in the world, 95 per cent of the total coconut production in the country is made into copra (Vibar, 1972). The meal left after the extraction of oil from copra contains approximately 20-25 per cent protein, depending upon the residual oil content.

However, due to unsanitary copra processing practices, this product is unfit for human consumption. Its high fiber content, 9 to 11 per cent, also limits its use as an effective protein supplement, even when prepared under conditions which render it suitable for human use.

Efforts therefore have been undertaken to process fresh coconuts to recover oil and food grade protein. Loo (1968) reviewed the status of coconut protein concentrate development studies. Early processes developed have been marked by inefficient recovery of oil and protein and have therefore not been used in the commercial production of protein concentrate. Recent studies on the aqueous processing of fresh coconuts were directed towards the efficient recovery of oil and coconut skin milk (Hagenmaier *et al,* 1973). He reported that the skim milk fraction contains 69 per cent of the total protein, 83 per cent of the salts and 97 per cent of the soluble carbohydrates of the coconut. Gonzales *et al* (1972) developed a process for producing canned coconut cream, leaving the skim portion as a by-product of the process.

The aqueous phase has been spray dried (Hagenmaier *et al.* 1973) or concentrated under vacuum to 60° Brix into "honey". The proteins have also been precipitated at the isoelectric point of coconut protein (pH 4-4.5) or by heat coagulation at 80-82° C. (Sreenivasan, 1963). Acid adjustment or heat coagulation produces a creamy white, bland protein concentrate (by definition containing a minimum of 70 per cent protein on a moisure-free basis) found suitable a protein ingredient in a wide variety of foods. It has been used in the preparation of weaning foods (Sreenivasan, 1963; Payumo *et al*, 1970); baked goods (Alabastro *et al,* 1969); beverage (Guerrero *et al*, 1973) and canned simulated meat products (Payumo *et al,* 1971; 1973).

The objective of this investigation was to stdy the suitability of heat coagulated coconut protein concentrate in the preparation of dehydated textured protein food. The chemical and microbiological composition, nutritional value and storage life of the finished product were determined. It was also evaluated for use as a component in recipes in place of meat.

MATERIALS AND METHODS

Preparation of coconut protein concentrate

A simple flow diagram for the process is shown in Figure 1. The coarsely grated coconut meat was passed twice through a Ritz disintegrator to reduce particle size and permit better extraction of milk. Screen sieve No. 1-16 was used in the first passing and No. 032 for the second pass. Water (1 : 1) was added to the coconut meat after the first passing before putting it back into the disintegrator. Coconut milk was extracted by hand pressing, then squeezing, in the absence of a mechanical extractor or press. The milk was filtered through fine nylon cloth to remove the residue and then centrifuged in a three-phase de Laval centrige to separate the cream form the skin or aqueous portion. The skim milk was heated to a temperature of 80-85° C, maintained at that temperature for 30 minutes to coagulate the protein. The protein coagulate was filtered through fine nylon screen to separate the protein curd from the whey.

Product formulation

The heat coagulated protein concentrate was formulated into dehydrated textured product by the following method: To every 500 grams of coconut protein concentrate (80 per cent moisture), the following ingredients were added: 40 g. mung bean (*Phaseolus aureus*. Roxb.) flour; 5 g. food yeast, 9 g. agar; 4 g. salt; 28 ml vegetable oil; 50 ml. vegetable protein hydrolyzate; 30 drops synthetic meat flavor and 35 drops oleoresin paprika. The yeast was dissolved in 60 ml. of lukewarm water before adding to the mixture. The mixture was thoroughly blended, placed in a wide flat pan and allowed to stand for one hour at room temperature (27-30° C). The product was then steamed for 30 minutes, cut into

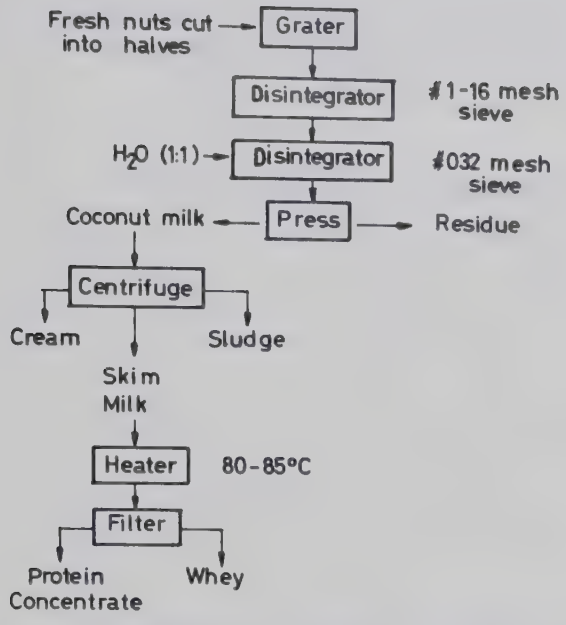


Fig. 1.–Simplified flow diagram for laboratory scale processing of fresh coconut for protein.

approximately 1/2 inch cubes and dried in a forced draft cabinet dryer et al 70-80° C for about 7 hours to a moisture level of about 5-7 per cent. Granules were prepared by passing the precooked mixture through a coarse meat grinder before drying.

The dried products were packed in aluminum foil packets of 100 grams each for storage at room temperature for a period of six months.

Analytical methods

Proximate analysis of the freshly prepared coconut protein concentrate and of the dehydrated textured protein products was performed using the standard AOAC methods (AOAC, 1970). The dehydrated product was further analyzed for the following: calcium, as described in AOAC (1970); phosphorus, using the modification of the Fiske and Subbarow method as described by Lowry and Lopez (1946); iron, by the method described by Hahn (1945); thiamine, by the thiochrome method of Hennessey and Cerecedo reported by Munsell *et al* (1949); niacin, by the USP microbiological method (1950) and riboflavin, by the Hodson and Norris (1939) fluorometric method.

Amino acid analysis was undertaken in a Model 120-C Beckman amino acid analyzer. No analysis was made for tryptophan.

Microbiological examination was done using standard bacteriological procedures for food (APHA, 1958).

Nutritional evaluation

Biological tests were performed with albino rats at the Biological Assay Branch of the Nutrition Research Laboratory, Food and Nutrition Research Center. The bio-assays done were adopted from the methods described by the National Academy of Science, National Research Council (1963). The criteria for determining the nutritive value of the proteins were protein efficiency ratio (PER), biological value (BV), coefficient of true digestibility (TD) and net protein utilization (NPU). The BV and TD were determined by the balance sheet method and the NPU by carcass analysis.

Rehydration capcity of the dehydrated products

Estimates of the rehydration capacity of the dehydrated products in cube and granular forms were done by soaking 10 g samples in either tap water or boiling water for varying periods of time, namely 10, 20, 30 and 60 minutes in a covered container. The samples were drained, wiped with absorbent tissue and weighed again after each soaking time. The amount of water absorbed was calculated by substracting the initial weight from the final weight of the sample.

Sensory evaluation of recipes

Sensory tests were conducted by a six member panel of trained tasters using a nine-point hedonic scale (1-dislike extremely up to 9-like extremely). The dehydrated cubes and granules were rehydrated

before cooking into a local sauteed vegetable recipe and omelet, respectively. They were used in place of meat in the recipes. The data obtained was statistically analyzed by ANOVA to ascertain the significance of the results.

RESULTS AND DISCUSSION

Properties and yield of protein concentrate

Heat coagulated coconut protein concentrate was creamy white in color, bland in flavor with a soft texture similar to cottage cheese. An average yield of 65 grams of protein concentrate was obtained from 1 kilogram of fresh coconut meat. Table 1 shows the proximate composition of the heat coagulated protein. Data obtained is similar to that reported by Sreenivasan (1963) except for the residual oil content of the concentrate. By commonly accepted definition of a protein concentrate (i. e., 70 per cent on a moisture-free basis), the product could be more correctly referred to as a protein concentrate instead of an isolate.

TABLE 1

Proximate composition of heat coagulated coconut protein concentrate

Nutrient	Percent
Moisture, g.	79.7
Protein, g (N X 6.25).	14.4 (70.9)*
Fat, g.	3.1
Ash, g.	1.0
Crude fiber, g.	0.1
Carbohydrates (by diff).	1.8

*Value on a moisture-free basis.

Evaluation of the textured protein products

The dehydrated textured products were brown in color with a slightly porous texture. A slightly open, spongy texture was found necessary for faster absorption of water, although a highly porous product was disliked by the panel and described as not characteristic of meat texture. The hydration characteristics of the two types of products are shown in Figure 2. The dehydrated cubes absorbed about equal their weight of water when soaked for 60 minutes in either tap or hot water; the ground samples, almost two-and-one half to three times their weight in tap or hot water, respectively, for the same length of time. However, hydration capacity was almost maximum at twenty minutes. Water absorption after this period was considered insignificant, for practical purposes. Because of smaller size and greater exposed surface, the granular form absorbed water faster than the cube form. Likewise, soaking in hot water resulted in faster absorption than tap water for both types.

The chemical composition of the two types of dehydrated products are shown in Table 2. Results showed that the products contain approximately 52 per cent protein (D. W. basis) and fairly high values for calcium, phosphosurs, thiamine and iron. The protein content is comparable to that of pork tenderloin, a meat portion commonly used in Filipino recipes. The fat content of the product can be properly adjusted depending on the needs of the target consumer.

Amino acid analysis of the textured protein product is shown in Table 3. As compared with the FAO reference pattern, the major limiting amino acids are the

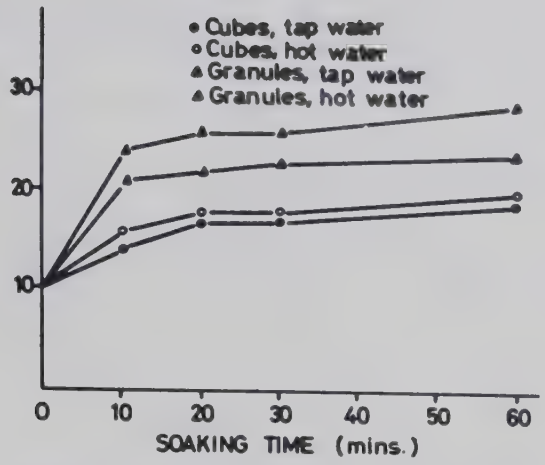


Fig. 2.–Hydration capacity of dehydrated textured protein products.

TABLE 2

Chemical analysis of dehydrated textured protein products compared with pork tenderloin

Nutrients per 100 g	Cubes	Granules	Pork tenderloin[a]
Moisture, g.	7.9	4.6	6.0
Protein, g (N X 6.25).	48.3 (52.4)	49.6 (52.0)	42.9
Fat, g.	13.7	18.7	48.5
Ash, g.	10.2	10.3	2.6
Crude fiber, g.	0.6	0.7	–
Carbohydrates, g (by diff).	19.9	16.8	0.0
Calcium, mg.	192	197	36
Phosphorus, mg.	664	685	403
Iron, mg.	4.1	4.4	3.8
Thiamine, mg.	20.2	18.2	1.8
Riboflavin, mg.	1.5	1.4	0.7
Niacin, mg.	1.5	1.4	13.6

( ) Value in parenthesis computed on a dry weight basis.

[a] Data were taken from "Food Composition Table Recommended for Use in the Philippines". Handbook I, 4th rev. FNRC, NIST, NSDB, Manila, Phils. 1968. Computed to 6 % moisture.

sulfur-containing amino acids.

Microbiological analysis showed the following results: Total plate count-533 colonies/g; negative for yeasts and mold, staphylococcus, coliform, salmonella and thermophiles. The process appeared to yield a sterile product. The dehydrated textured food meets the acceptance criteria for dried protein foods set by the Protein Advisory Group of the United Nations (1972).

Biological evaluation of the product showed the following results: Protein efficiency ratio (PER) - 1.8 (corrected for casein - 2.5); Biological Value - 57; True digestibility - 87 and Net Protein Utilization - 57. The PER value conforms with that established by USDA (1971) for textured vegetable protein which must not be less than 1.8 on the basis of PER - 2.5 for casein. The nutritional value of the product was probably lowered by its low methionine-cystine content.

Results of the sensory evaluation of recipes utilizing the two types of textured

TABLE 3

Amino acid conposition of textured vegetable protein compared with FAO reference pattern

Amino acid mg/g protein	Textured vegetable protein	FAO reference pattern
Isoleucine.	42	40
Leucine.	63	70
Lysine.	46	55
Methionine plus cystine. . . .	11	35
Phenylalanine plus tyrosine. .	76	60
Threonine.	28	40
Tryptophan.	–	10
Valine.	42	50

TABLE 4

Mean acceptability scores of recipes prepared from dehydrated textured vegetable protein cubes and granules on storage

Period of storage (months)	Mean acceptability score	
	Vegetable Macedoine[a]	Omelet[b]
0	8.3±0.18	7.8±0.31
1	7.7±0.21	8.7±0.21
2	8.0±0.37	8.3±0.21
3	7.0±0.31	7.8±0.17
4	7.3±0.21	8.0±0.26
5	7.2±0.31	8.4±0.22
6	7.5±0.21	7.8±0.48

[a] Cube type used in place of meat.
[b] Granular type used in place of meat.

protein product are shown in Table 4. Evaluation was done monthly from 0 to 6 months. In both recipes, the dehydrated stored products were rehydrated in water by soaking in boiling water for 30 minutes and in tap water for 10 minutes for the cubes and granules, respectively. The textured protein products were used in place of meat in the recipes. Storage had no adverse effects on the acceptability of the samples. There were no significant differences at 1 % level of significance on the acceptability scores during the six months storage period. The dehydrated textured products may therefore be considered as an acceptable substitutes for meat in meat-containing recipes. There was slight preference, however, for the omelet recipe.

The study shows that dehydrated textured vegetable protein products can be prepared from coconut protein concentrate as the basic ingredient with relatively very simple and inexpensive equipment. Inasmuch as the main objective of this investigation was the production of a meat substitute from a bland coconut protein product, the overall efficiency of the heat coagulation process was not studies. A

more efficient process can certainly be achieved by undertaking a more critical study of the various extraction and coagulation procedures involved.

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The evaluation of the nutritive value and quality in fresh *brassicaceous* vegetables after harvest, during preparation, and subsequent to storage

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ABSTRACT

Most *Brassicaceous* vegetable crops are hardy and grow profusely in cool weather and in all types of soil, but fertile, heavy soils having high-moisture retention are the most desirable. Some of these crops are important in home gardens, therefore, they have a place in urbanized population centers. Likewise, some of them appear to be beautiful and colorful plants that have ornamental values in addition to nutritional benefits. The world is faced with a need for highly nutritive food. New directions and an increased consciousness in nutrition will have a significant impact upon modern agriculture and the food industry. The main purpose of this paper is to present the high nutritive quality of *Brassicaceous* vegetables and their ability to fulfill the consumer's needs for vitamins, minerals, and proteins.

INTRODUCTION

The *Brassica* genus of the cruciferae family provides a notable group of vegetables such as cabbage (*Brassica oleracea* L. var. *capitata* L.), cauliflower (*B. oleracea* L. var. *botrytis* L.), kale and collard (*B. oleracea* L. var *acephala* D. C.), Brussels sprout (*B. oleracea* L. var. gemmifera Zenker), sporuting broccoli (*B. oleracea* L. var. *italica* Plenk), kohlrabi (*B. caulorapa* Pasq.), turnip (*B. rapa* L.), rutabaga (*B. napobrassica* Mill.), Chineses cabbage: pe-tsai (*B. pekinensis* Rupr.), pak-choi (*B. chinensis* L.), and mustard (*B. juncea* Coss; *B. hirta* Moench).

Most *Brassicaceous* vegetable crops are hardy, and grow profusely in cool weather and in all types of soil, but fertile, heavy soils having high-moisture retention are the most desirable. Since the crops of this genus are closely related, their cultural requirements and susceptibility to several diseases and insects are similar.

Some of these crops are important in home gardens; therefore, they have a place in urbanized population centers. Likewise, some of them appear to be

beautiful and colorful plants that have ornamental values in addition to nutritional benefits.

The world is faced with a need for highly nutritive food. New directions and an increased consciousness in NUTRITION will have a significant impact upon modern agriculture and the food industry.

The main purpose of this paper is to assess the nutritive quality of *Brassicaeous* vegetables to fulfill the consumer's needs for vitamins, minerals, proteins, and calories. The data presented here may assist in decisions as to the relative importance of these vegetables when compared with other commodities. Major fruits and vegetables are evaluated according to their composition and the dietary needs of man.

NUTRIENTS AND QUALITY OF *BRASSICACEOUS* VEGETABLES

As the vegetables undergo transportation and storage en route to the consumer's table, they suffer losses in quantity, quality, and particularly nutritive values which are frequently destroyed or denatured.

HORTICULTURAL CONSIDERATIONS

The nutrient content of fresh vegetables depends upon factors such as cultivars, environment, and maturity, and is synthesized in a plant or in its parts during growth.

Cultivars

In the past, geneticists and plant breeder were concerned with breeding new cultivars primarily to achieve an increased yield and disease resistance and/or to enhance color, flavor, and texture; and in most breeding programs, nutritive value had often been merely an afterthought. However, this attitude is now changing. The recent withdrawal of the 'Lenape' cultivar of potatoes by the U. S. and Canadian Departments of Agriculture due to its unusually high concentrations of solanine, and the Food and Drug Administation's requirement that new cultivars meet nutritional and toxicant requirements in order to be classified 'GRAS' (Generally Recognized As Safe), mean that plant breeders must become more actively concerned with the nutritive composition of their breeding lines.

Brell et al. (1940) found a 35-fold range in the ascorbic acid content of 31 cultivars of cabbage. Walker and Foster (1946) concluded that selection and genetic manipulation could increase the ascorbic acid content of vegetables considerably.

Environment

Some ecological, cultural, and physiological factors significantly influence the chemical and nutritional composition and anatomical and morphological structure of plants.

Light. Hamner and Parks (1944) found 28.2 mg ascorbic acid in 100 grams of turnip greens after a 200-foot-candle light exposure and 235 mg per 100 grams after exposure to 5,000 foot candles. Reder et al. (1943 b) studied three crops of turnip greens and found 191 mg of ascorbic acid per 100 gram samples when plants were grown in the sunshine during 49 % of the growing season and only 128 mg in those grown in a limited amount of sunshine. Somers and Kelly (1957) reported that turnips grown in full sunlight contained more ascorbic acid and less carotene than those grown in the shade. Turnips harvested in the morning have been reported to

contain more riboflavin than those harvested at other times of the day. Thiamine synthesis in plants is stimulated by light and generally occurs in the leaves, and as a rule, increases in concentration until the plant is mature. Light stimulates plant growth which in turn increases the demand of iron.

Temperature. The optimum temperature for rapid growth by a species of edibl plant does not always equate with its optimum for synthesizing and accumulating nutrients. Furthermore, the specific temperature promoting the greatest translocation, synthesis, and accumulation of one nutrient is often different for another. In general, the content of ascorbic acid in turnip greens held in the dark decreased as the temperature increased from 50° to 86° F (Somers et al., 1948).

Gustafson (1950) noted a higher riboflavin content in broccoli and cabbage grown at 50° to 59° F than at other temperatures, while broccoli and cabbage had the highest thiamine content when they were grown at 50° and 59° F.

Season and climate. Pooles et al. (1944) and Smith et al. (1948) reported increases in the quantity of ascorbic acid in cabbage as the growing season progressed. On the other hand, according to Pyke (1942), cabbage harvested early in the season had five times more ascorbic acid than cabbage harvested late. Hansen (1945) reported that climatic changes did not affect the ascorbic acid values of broccoli, collard, and kale.

As the literature concerning season and climate is limited and conflicting, it is difficult to draw a definite conclusion as to the relation of season to the nutrient content.

Location, soil fertility, and irrigation. The ascorbic acid content of turnips (Reder et al., 1943 a), cabbage (Janes, 1944), and rutabaga (Nylund, 1949) were influenced by the locations of the crops which were no doubt influenced by variations in soil fertility and moisture. Aberg and Ekdahl (1948) found less ascorbic acid in kale with suboptimal nitrogen than when adequately fertilized. Burrell et al. (1940), working with cabbage, and Sheets et al. (1954), with collard, observed that ascorbic acid content increased with nitrogen fertilization. Sheets et al. (1954), however, found that the carotene in collard increased because of nitrogen-deficient soil. In general, it is evident that nitrogen fertilization of soil markedly increases the crop yield but has only a mild or insignificant effect upon its nutrient content. The effects of calcium fertilization depend upon the pH of the soil. Hansen (1945) noted no effects from a limestone treatment on the calcium content of kale, whereas Maynard et al. (1964) noted a significant increase. Sheets et al. (1944) conducted an extensive study of liming and observed a slight increase in calcium in turnips, but found no significant differences in the calcium content in collard following liming (Sheets et al., 1954). Plants grown in soil with a high concentration of sulfate contained higher quantities of both inorganic and organic sulfur than those grown with low concentrations.

Eheart (1966) noted that the applicatin of nitrogen at the rate of 100 pounds per acre decreased chlorophyll *a*, total chlorophyll, and carotene in broccoli. Eheart et al. (1955) had previously shown that: the ascorbic acid content of turnip greens was highest in the spring and lowest in the fall; carotene content decreased with high light intensity; thiamine content increased when minor elements were included in the fertilizer; and riboflavin content, which also increased with high light intensity, evidenced an additional increase when the light was combined with an application of trace elements to the soil. Holmes et al. (1945) reported that the addition of 150 pounds of magnesium sulfate per acre significantly increased the

magnesium content of kale and slightly increased its calcium and phosphorus. Limestone applied at the rate of 1,000 pounds per acre increased the calcium and phosphorus contents of the kale but depressed its iron content. Neither magnesium sulfate nor limestone had any significant effects on the carotene contents.

Del Valle and Harmon (1967) showed that turnip growth was influenced by soil temperature because of the increased uptake of minerals and the phosphorus and potassium contents were higher in leaves when large quantities of minerals were available in the soil. They further indicated that the calcium and phosphorus contents of collard were increased by soil temperatures of 75° and 85° F and decreased at 95° F. Sheets e al. (1955) stated that the phosphorus and carotene contents increased in turnip greens as irrigaion increased.

The literature relating to the effects of fertilization upon the nutrient contents is voluminous and generally contradictory, probably because of variations in soil chemistry, water availability, and other environmental factors.

Maturity

Small cabbage heads are higher in ascorbic acid than large ones (Branion et al., 1948), and outer leaves of cabbage contain 2 to 4 times more iron than the stem, 1.5 to 3 times as much iron, 1.5 times as mch ascorbic acid, and 21 times as much carotene in the more mature outer green leaves as in the inner bleached leaves (Sheets et al., 1941). Speirs et al. (1951) noted that small turnip leaves had more thiamine and riboflavin than ascorbic acid, but less carotene than large leaves. In general, broccoli, cabbage, and cauliflower had the maximum content of nutritive value at harvest time.

It is exceedingly important that vegetables in general and crucifers in particular be harvested at the proper time. The exact degree of matyrity at which a given cultivar or species should be harvested depends upon the purpose for which it is used. When a cabbage or a cauliflower head is harvested for the local fresh market, it must be harvested at prime maturity. When it is harvested for shipping or processing, it should be harvested a week earlier. If it is harvested too early and then stored for a length of time, it may shrivel and the characteristic aroma will disappear. If harvested too late and then stored for a longer period, it might have higher than average concentrations of sulfur compounds and less ascorbic acid and other water-soluble vitamins. Explicit directions and measurements cannot be provided as to the best time to harvest *Brassicaceous* vegetables. No one index of maturation is infallible, and often a combination of several indices and, above all, experience is essential.

PRODUCTION (TONNAGE AND NUTRIENTS) CONSIDERATION

Horticultural commodities are generally bought and consumed primarily for flavor and appearance rather than for nutritive value. This concept must change if the burgeoning world population is to be properly fed. This report contains certain basic information pertaining to the nutritive contribution of 12 major fruits, 12 cruciferous predominantly *Brassicaceous* vegetables, and 18 other major vegetables (Table 1)

Yield

Food that are produced and consumed in large quantities such as potatoes, tomatoes, and oranges can significantly contribute to the well-being of a nation by supplying calories and a certain amount of some nutrients even though their relative

TABLE 1

Composition of forty-two fresh fruits and vegetables per 100 gm edible portion

No. Food	Water (%)	Energy (Cal.)	Protein (%)	Fat (%)	Carbo-hydrate (%)	Calcium (mg)	Phos-phorus (mg)
1. Apricot.	85.3	51(13)	1.0(32)	.2(28)	12.8(12)	17(29)	23(30)
2. Peach.	89.1	38(21)	.6(38)	.1(38)	9.7(17)	9(36)	19(34)
3. Orange.	86.0	49(14)	1.0(31)	.2(27)	12.2(13)	41(14)	20(33)
4. Grapefruit.	88.4	41(20)	.5(40)	.1(39)	10.6(15)	16(30)	16(38)
5. Plum.	81.1	66(8)	.5(41)	.2[a](29)	17.8(5)	18(28)	17(36)
6. Grape.	81.6	69(7)	1.3(21)	1.0(1)	15.7(7)	16(32)	12(39)
7. Sour Cherry.	83.7	58(11)	1.2(25)	.3(17)	14.3(11)	22(26)	19(35)
8. Apple.	84.4	58(10)	.2(42)	.6(5)	14.5(9)	7(40)	10(42)
9. Strawberry.	89.9	37(23)	.7(36)	.5(6)	8.4(21)	21(27)	21(32)
10. Watermelon.	92.6	26(33)	.5(39)	.2(21)	6.4(27)	7(39)	10(41)
11. Pear.	83.2	61(9)	.7(37)	.4(10)	15.3(8)	8(37)	11(40)
12. Banana.	75.7	85(4)	1.1(30)	.2(30)	22.2(2)	8(38)	26(29)
13. Carrot.	88.2	42(19)	1.1(28)	.2(26)	9.7(18)	37(16)	36(16)
14. Sweet Potato.	70.6	114(2)	1.7(17)	.4(12)	26.3(1)	32(19)	47(16)
15. Tomato.	93.5	22(36)	1.1(27)	.2(19)	4.7(35)	13(34)	27(27)
16. Sweet Corn.	72.7	96(3)	3.5(7)	1.0(2)	22.1(3)	3(42)	111(3)
17. Pepper.	93.4	22(35)	1.2(24)	.2(20)	4.8(34)	9(35)	22(31)
18. Lettuce.	95.1	14(41)	1.2(22)	.2(18)	2.5(42)	35(18)	26(28)
19. Potato.	79.8	76(6)	2.1(14)	.1(42)	17.1(6)	7(41)	53(12)
20. Squash.	94.0	19(38)	1.1(26)	.1(34)	4.2(37)	28(20)	29(23)
21. Onion.	89.1	38(22)	1.5(19)	.1(37)	8.7(20)	27(21)	36(20)
22. Cucumber.	95.1	15(40)	.9(33)	.1(31)	3.4(39)	25(23)	27(26)
23. Spinach.	90.7	26(32)	3.2(8)	.3(14)	4.3(36)	93(7)	51(14)
24. Lima Bean.	67.5	123(1)	8.4(1)	.5(8)	22.1(4)	52(10)	142(1)
25. Pea.	78.0	84(5)	6.3(2)	.4(11)	14.4(10)	26(22)	116(2)
26. Asparagus.	91.7	26(31)	2.5(12)	.2(23)	5.0(32)	22(25)	62(8)
27. Cantaloupe.	91.2	30(27)	7(35)	.1(35)	7.5(23)	14(33)	16(37)
28. Snap Bean.	90.1	32(24)	1.9(16)	.2(24)	7.1(25)	56(9)	44(17)
29. Beet.	87.3	43(18)	1.6(18)	.1(40)	9.9(16)	16(31)	33(22)
30. Celery.	94.1	17(39)	.9(34)	.1(33)	3.9(38)	39(15)	28(25)
31. Cabbage.	92.4	24(34)	1.3(20)	.2(22)	5.4(30)	49(11)	29(24)
32. Broccoli.	89.1	32(25)	3.6(6)	.3(16)	5.9(28)	103(6)	78(7)
33. Brussels Sprout.	85.2	45(17)	4.9(4)	.4(9)	8.3(22)	36(17)	80(6)
34. Cauliflower.	91.0	27(30)	2.7(11)	.2(25)	5.2(31)	25(24)	56(10)
35. Kale.	82.7	53(12)	6.0(3)	.8(4)	9.0(19)	249(2)	93(4)
36. Watercress*.	93.3	19(37)	2.2(13)	.3(13)	3.0(41)	151(5)	54(11)
37. Mustard green.	89.5	31(26)	3.0(10)	.5(7)	5.6(29)	183(4)	50(15)
38. Turnip green.	90.3	28(29)	3.0(9)	.3(15)	5.0(33)	246(3)	58(9)
39. Chinese Cabbage.	95.0	14(42)	1.2(23)	.1(32)	3.0(4)	43(12)	40(18)
40. Rutabaga.	87.0	46(15)	1.1(29)	.1(41)	11.0(14)	66(8)	39(19)
41. Collard.	85.3	45(16)	4.8(5)	.8(3)	7.5(24)	250(1)	82(5)
42. Kohlrabi.	90.3	29(28)	2.0(15)	.1(36)	6.6(26)	41(13)	51(13)

Main source: Agricultural Handbook No. 8, USDA 1963, Washington, D. C.; [a] The Heinz Handbook of Nutrition (1959), McGraw-Hill Book Company, New York.

Iron (mg)	Sodium (mg)	Po-tassium (mg)	Magnes-ium (mg)	Vita-min A (IU)	Thia-mine (mg)	Ribo-flavin (mg)	Niacin (mg)	Vita-min C (mg)
.5(30)	1(40)	281(18)	12(34)	2,700(10)	.03(39)	.04(33)	.6(24)	10(31)
.5(29)	1(37)	202(32)	10(38)	1,330(12)	.02(41)	.05(24)	1.0(10)	7(38)
.4(37)	1(39)	200(33)	11(37)	200(29)	.10(12)	.04(32)	.4(30)	50(13)
.4(35)	1(38)	135(39)	12(33)	80(35)	.04(32)	.02(41)	.2(40)	38(16)
.5(31)	2(31)	299(15)	9(39)	300(25)	.08(19)	.03(40)	.5(28)	5[a](39)
.4(39)	3(26)	158(37)	13(30)	100(33)	.05(29)	.03(39)	.3(36)	4(42)
.4(38)	2(29)	191(34)	14(28	1,000(13)	.05(28)	.06(20)	.4(31)	10(32)
.3(41)	1(41)	110(41)	8(41)	90(34)	.03(40)	.02(42)	.1(41)	4(40)
1.0(15)	1(36)	164(35)	12(31)	60(37)	.03(35)	.07(17)	.6(22)	59(11)
.5(26)	1(35)	100(42)	8(40)	590(19)	.03(35)	.03(37)	.2(38)	7(37)
.3(42)	2(30)	130(40)	7(42)	20(41)	.02(42)	.04(34)	1(42)	4(41)
.7(20)	1(42)	370(10)	33(10)	190(30)	.05(30)	.06(21)	.7(18)	10(33)
.7(18)	47(6)	341(12)	23(18)	11,000(1)	.06(24)	.05(25)	.6(23)	8(36)
.7(22)	10(19)	243(27)	31(12)	8,800(4)	.10(15)	.06(22)	.6(25)	21(24)
.5(25)	3(25)	244(25)	14(27)	900(15)	.06(22)	.04(29)	.7(16)	23(22)
.7(21)	10[b](18)	280(19)	48(5)	400(24)	.15(7)	.12(11)	1.7(4)	12(27)
.7(17)	13(14)	213(30)	18(22)	420(22)	.08(16)	.08(16)	.5(26)	128(4)
2.0(5)	9(20)	264(21)	11(36)	900(14)	.06(21)	.06(19)	.3(32)	8(35)
.6(24)	3(27)	407(4)	34(9)	20[a](42)	.10(14)	.04(35)	1.5(6)	20(25)
.4(32)	1(34)	202(31)	17(23)	410(23)	.05(26)	.09(15)	1.0(9)	22(23)
.5(28)	10(17)	157(38)	12(32)	40(38)	.03(37)	.04(31)	.2(39)	10(29)
1.1(11)	6(23)	160(36)	11(35)	250(27)	.03(33)	.04(27)	.2(37)	11(28)
3.1(1)	71(3)	470(2)	88(1)	8,100(5)	.10(10)	.20(7)	.6(21)	51(12)
2.8(3)	2(33)	650(1)	67(2)	290(26)	.24(2)	.12(12)	1.4(7)	29(19)
1.9(6)	2(32)	316(14)	35(8)	640(17)	.35(1)	.14(10)	2.9(1)	27(20)
1.0(14)	2(28)	278(20)	20(21)	900(16)	.18(4)	.20(6)	1.5(5)	33(17)
.4(34)	12(16)	251(23)	17[b](24)	3,400(9)	.04(31)	.03(38)	.6(20)	33(18)
.8(16)	7(22)	243(26)	32(11	600(18)	.08(18)	.11(13)	.5(27)	19(26)
.7(19)	60(4)	335(13)	25(15)	20(40)	.03(38)	.05(26)	.4(29)	10(30)
.3(40)	126(1)	341(11)	22(19)	240(28)	.03(34)	.03(36)	.3(33)	9(34)
.4(33)	20(11)	233(29)	13(29)	130(32)	.05(27)	.05(23)	.3(34)	47(14)
1.1(13)	15(12)	382(6)	24(17)	2,500(11)	.10(11)	.23(4)	.9(12)	113(5)
1.5(10)	14(13)	390(5)	29(13)	550(21)	.10(13)	.16(9)	.9(13)	102(6)
1.1(12)	13(15)	295(16)	24(16)	60(36)	.11(8)	.10(14)	.7(17)	78(9)
2.7(4)	75(2)	238(7)	37(7)	10,000(2)	.16(6)	.26(3)	2.1(2)	186(1)
1.7(8)	52(5)	282(17)	20(20)	4,900(8)	.08(17)	.16(8)	.9(11)	79(8)
3.0(2)	32(9)	377(8)	27(14)	7,000(7)	.11(9)	.22(5)	.8(15)	97(7)
1.8(7)	40[b](7)	250[b](24)	58(3)	7,600(6)	.21(3)	.39(1)	.8(14)	139(3)
.6(23)	23(10)	253(22)	14(26)	150(31)	.05(25)	.04(28)	.6(19)	25(21)
.4(36)	5(24)	239(28)	15(25)	580(20)	.07(20)	.07(18)	1.1(8)	43(15)
1.5(9)	40[b](8)	450(3)	57(4)	9,300(3)	.16(5)	.31(2)	1.7(3)	152(2)
.5(27)	8(21)	372(9)	37(6)	20(39)	.06(23)	.04(30)	.3(35)	66(10)

[b] **Nutrition Composition of Fresh California-Grown Vegetables (1962), California Agricultural Experiment Station Bulletin No. 788, Davis, California. Numbers in Parenthesis denote relative ranking for each nutritive attribute among 42 fruits and vegetables; 1 = highest and 42 = lowest amount.**

nutritive value is not high. However, a definite problem of balance does exist in the national diet. Althoug potatoes rank No. 1 among the 42 fruits and vegetables surveyed for this paper in annual production of riboflavin, a person would have to eat about 6.5 pounds per day to meet the recommended daily requirement for an adult. In 1970, the production of potatoes, the most prevalent horticultural crop, was about 16.5 million tons; that of oranges, 8 million tons; and tomatoes, 6 million tons; while less than 1.5 million tons of cabbage, an excellent source of total nutrients, were harvested. Cabbage is the only *Brassicaceous* vegetable listed among the leading 15 fruits and vegetables (Figure 1). In the last 25 years, however, cabbage acreage has progessively declined; consequently, the per capita consumption in 1970 was less than half that in 1944 (USDA, Agricultural Statistics, 1972).

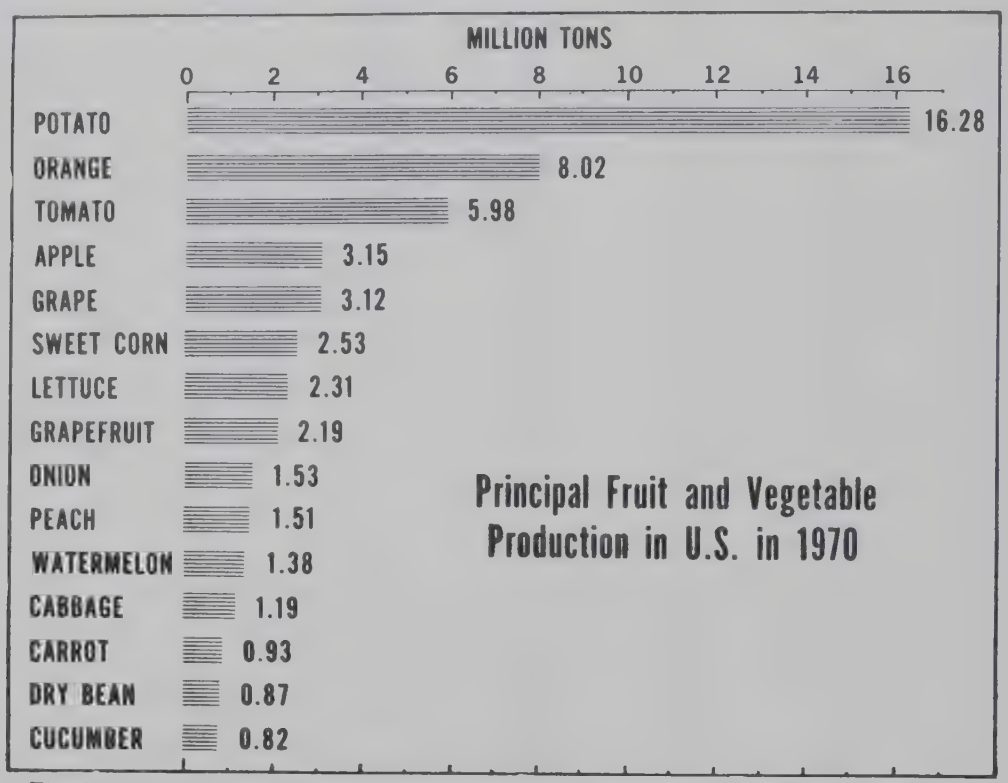


Fig. 1.–Yield of the 15 most prevalent fruits and vegetables in the United States of America in 1970.

Consumption

The extent of which a food contributes nutrients to man's total food supply is governed both by the AMOUNT OF NUTRIENTS and the QUANTITY OF THE FOOD that is consumed. Taking both of these factors into consideration, calculations have been made on the proportions of certain nutrients in the food supply that are attributable to fruits and vegetables. Such information for the year 1971 from the National Food Situation, November, 1971, is summarized in Figure 2 which shows total and relative percentages of the nutrients in the food supply that are derived from fruits and vegetables. Per capita consumption of fruits and vegetables in the United States in 1971 was about 530 pounds.

The United States Department of Agriculture Handbook No. 8 (1963), Agricultural Statistics (1972), and other pertinent references were used to assess the nutritive value of 42 fruits and vegetables. Each commodity was then ranked for each nutrient from 1 = the highest to 42 = the lowest. These numbers are presented in parentheses in the tables.

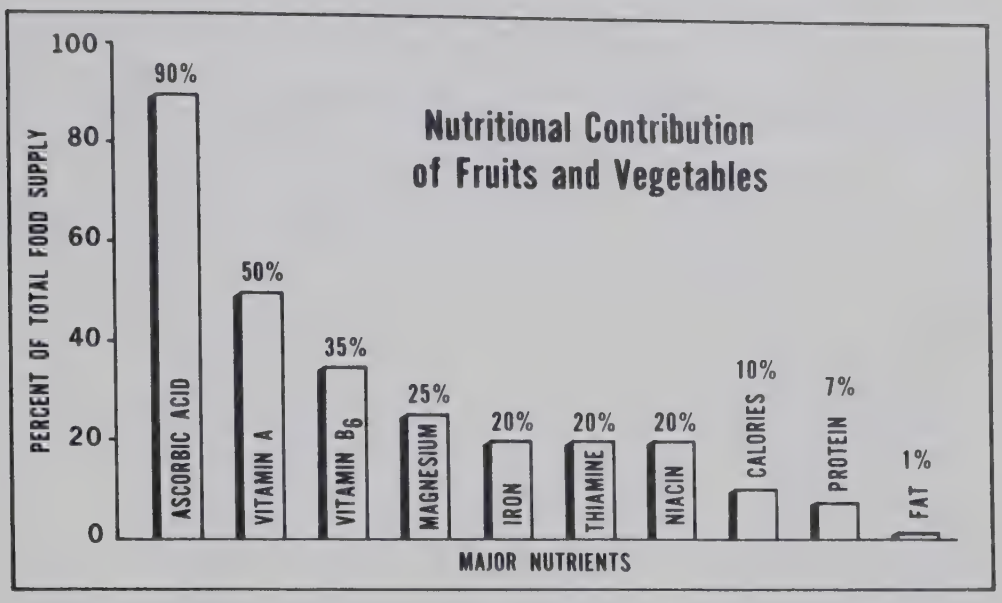


Fig. 2.–Nutrient contribution of fruits and vegetables compared to the percent of total food supply in 1971.

Energy: fats and carbohydrates

It is apparent from Table 1 that most of the *Brassicaceous* vegetables are low in calories, fats, and carbohydrates. The contribution to calories is mainly due to fats and fatty acids and the predominant fatty acids in *Brassicaceous* vegetables are linoleic and linolenic. These fatty acids are essential for the human body.

Proteins: quantity, quality, and availability

Table 1 shows that *Brassicaceous* vegetables are rich sources of proteins among the fruits and vegetables surveyed. The *Brassicaceous* vegetables contain all the essential amino acids and particularly sulfur containing amino acids. The biological value, digestibility, and net protein utilization when compared with the richest source of plant proteins, peas, and beans, the *Brassicaceous* vegetables compare very favorably.

Minerals

Table 1 shows that *Brassicaceous* vegetables are an excellent source of minerals such as calcium, iron, magnesium, sodium, potassium, and phosphorus, and their availability for the human body is relatively high.

Vitamins

Table 1 shows that most of the *Brassicaceous* vegetables contain significant amounts of Vitamin A (carotene), riboflavin, niacin, thiamine, and ascorbic acid.

In general it seems from the composite table based upon rank correlation tests conducted on the data in Table 1 (Table 2) that the *Brassicaceous* vegetables are the most efficient in synthesizing high concentrations of proteins, amino acids, minerals, and vitamins, and they are low in calorie content.

The comparison of relative nutritive value and production data shows that Americans are cultivating and consuming very little of the vegetables that are best for them. Generally, cabbage, cauliflower, broccoli, Brussels sporuts, turnip greens, mustard greens, and kale, which rank high in nutritive value, are most disliked and the least consumed.

TABLE 2

Relative nutritional value of 42 fruits and vegetables presented in Table 1 for cumulative of 15 nutrients

Fruits and vegetables	Rank for relative nutritional value	Water (%)
*Kale.	1	82.7
*Collard.	2	85.3
Lima bean.	3	67.5
*Turnip green.	4	90.3
Pea.	5	78.0
*Mustard green.	6	89.3
*Brussels sprout.	7	85.2
Spinach.	8	90.7
*Broccoli.	9	89.1
Sweet corn.	10	72.7
Watercress.	11	93.3
Sweet potato.	12	70.6
Asparagus.	13	91.7
*Cauliflower.	14	91.0
Snap bean.	15	91.1
Other *Brassicaceous* vegetables		
Rutabaga.	18	87.0
Kohlrabi.	19	90.3
Cabbage.	23	92.4
Chinese cabbage.	24	95.0
Other major fruits and vegetables		
Potato.	17	79.8
Tomato.	27	93.5
Orange.	29	86.0
Apple.	41	84.4
Watermelon.	42	92.6

*** Brassicaceous vegetables among top 15 fruits and vegetables.**

Note: If expressed on a dry weight basis, the ranking could be improved in favor of Brassicaceous vegetables because of their higher water content.

FLAVOR AND PREFERENCE CONSIDERATIONS

Brassicaceous flavors

Along with nutritional attributes, flavor is one of the most important properties of food. It is the flavor which determines whether we like or dislike a particular food. The flavor causes either satisfaction or a negative reaction, with the latter being the subject of many gastronomical anecdotes.

The flavor of *Brassicaceous* vegetables depends upon the cultivar, maturity, season, horticultural practices, storage, handling, and method of cooking. According to Johnson et al. ((1971 a), Koning and Kracht (1929) reported the presene of hydrogen sulfide and metahnethiol in cabbage. Jensen et al. (1953), using paper chromatography, showed the presence of 2-propenyl isothiocyanate in both

white and red cabbage and 3-butenyl isothiocyanate in red cabbage. Clapp et al. (1959) detected four thioureas from volatile isothiocyanates.

Dateo et al. (1957) noted that cabbage contains thermolabile sulfur compounds which evolve sulfurous odors. The major volatile sulfur compounds (dimethyl disulfide and hydrogen sulfide) of cooked cabbage are derived from a precursor L-S-methylcysteine sulfoxide, a free amino acid. Maruyama (1970) indicated that dimethyl trisulfide was a major aroma component in cooked *Brassicaceous* vegetables and proposed that the strong, unpleasant aroma that is characteristic of over-cooked *Brassicaceous* vegetables is due to the gradual loss of pleasant volatible components and resultant unmasking of unpleasant sulfur components.

MacLeod and MacLeod (1970), assessing the close relation among flavors of cabbage, cauliflower, and Brussels sprouts, found that isothiocynates with allyl isothiocyanate comprised over 6 % of the total volatiles. Brussels sprouts have a stronger flavor (5 times more allyl cyanide) than cabbage. Chopping increases the release of dimethyl sulfide in both vegetables. However, the flavor profile of Brussels sprouts differs slightly from that of cabbage as chopping sporuts reduces their production of carbonyls and increases that of thioglucoside sinigrin (allyl cyanide and allyl isothiocyanate). Hingand Weckel (1964) in their studies noted that rutabaga flavor consisted of ammonia, isothiocynates, hydrogen sulfide, dimethyl sulfide, dimethyl disulfide, 2-phenethyl isothiocyanate, mercaptans, and acetaldehyde.

Food preference

An excellent survey was conducted by Einstein and Hornstein (1970) on food preferences using 207 food items and nearly 50,000 college students representing about 1 % of the college enrollment of the United States in 1966-67. The responses were collected on a regional basis subdivided as to sex, and reported as national and regional totals. The foods were ranked in terms of 'liked', 'disliked', and 'do not know'. Median and percentile for each food class as well as for all foods were calculated. The relationships between food preferences and nutritional values were examined. The percent of the recommended dietary allowance for vitamins A and C and minerals, calcium, and iron provided by one serving was calculated for each food. The results indicated that if food preferences were the sole determinant of food intake then the dietary intake would be low in vitamin A. Cabbage, cauliflower, Brussels sprouts, broccoli, turnips and kale were the least liked foods. Coleslaw, with its raw cabbage, was an exception. Similarly, Kamen et al.(1967) and Peryam and Seaton (1962) in surveys for the U. S. Army Quartermaster Corps of the food preferences of service men, found that vegetables in general and, *Brassicaceous* vegetables in particular, were disliked. Van Riter (1956) made a study in a college women's dormitory and found that strong-flavored vegetables such as turnips, broccoli, and cabbage were the least acceptable among 26 vegetables surveyed.

According to the Household Food Consumption Survey in 1965-66 (USDA, 1972) on the dark-green and deep-yellow vegetable group only 10 to 20 % of all the individuals contacted ate them. The consumption was significantly higher in the South than in the North, in the urban than in the rural areas, and in the under $3.000 than in the over $8.000 annual income class. The percentage of consumers eating these vegetables in restaurants was very low (about 4 %). More people (15 to 40 %) had diets below recommended allowances for calcium and iron than for other nutrients. In addition, these individuals ate significantly less (14 to 45 %) vitamin A, thiamine, riboflavin, ascorbic acid, vitamin B_6, magnesium and vitamin B_{12} than was required.

In general, *Brassicaceous* vegetables contain high amounts of objectionable sulfur compounds and have a mushy texture after extended boiling. They also undergo a significant loss of color from prolonged heating in the kitchens of restaurants or dormitories and are not preferred by the consumers despite their being excellent sources of protein, vitamins, and minerals, with few calories. Hence it becomes a challenge to make these vegetables more acceptable by reducing the sulfur compounds without losing the sulfur-containing amino acids and still maintain the crisp texture and attractive color during processing and prior to seving. Research must be geared to this end if these highly nutritious and low-caloried vegetables are to be made into more acceptable products. Market research has shown that the key factor in the success of new products or new forms of old products is not cost, nutrition, convenience, nor stability, it is the QUALITY as shown by the flavor, texture, and color of the food served at the table.

TOXICOLOGICAL CONSIDERATIONS

Vegetables of the *Brassica* genus contain goitrogens which cause enlargement of the thyroid glands. Natural thioglucosides are the source of goitrogens. The same thioglucosides with their associate enzyme(s) impart the desirable culinary flavor of cabbage, broccoli, and cauliflower. The thioglucosides (sinigrin), nongoitrogen, upon enzymatic hydrolysis, yield glucose and bisúlfate and allylthiocyanate-a goitrogen (Figure 3). The thioglucosides, progrotrin and epi-progoitrin, are the precursors of an antithyroid compound named goitrin (5-Vinyloxazolidine-2-thione). Goitrin is formed subsequent to hydrolysis and then through cyclization of an unstable isothiocyanate-containing hydroxyl group (Figure 4).

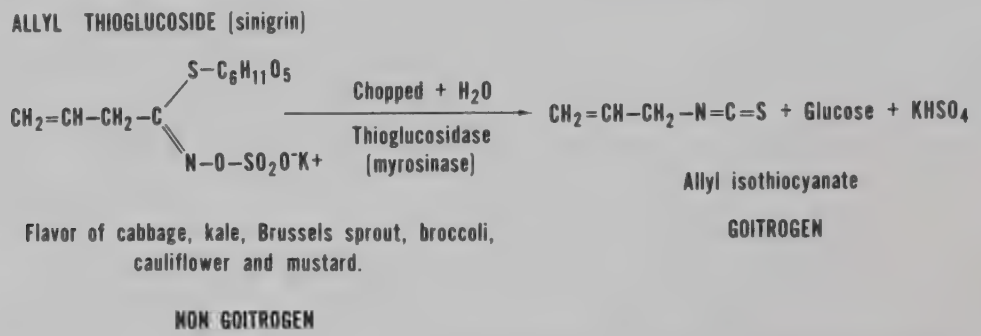


Fig. 3.–The enzymatic hydrolysis of allyl thioglucoside (sinigrin) to allyl isothiocyanate.

Greer (1956) found that most of the goitrogen properties of the product might be lost during cooking. However, they might be in significant concentrations in packaged cut coleslaw which is stored for days in the supermarket display cases.

Two *Brassicaceous* vegetables having high concentrations of thiocyanate are cauliflower and kale. However, a daily intake of about 20 pounds of cauliflower or kale would be required to furnish a goitrogenic concentration of thiocyanate in the blood.

Therapeutic and other pharmacological properties. Cheney (1942, 1949, 1950, 1952 , 1954, 1955) and Cheney et al. (1956) of the Stanford Medical School reported that a new entity which they termed vitamin U is an important constituent of cabbage. In a series of articles, he and his co-workers reviewed the raw cabbage juice therapy for peptic ulcers and indicated that an antipeptic ulcer dietary factor is present in fresh cabbage juice. This factor is readily destroyed by heat. A clinical study of patients indicated that concentrated cabbage juice is significantly effective

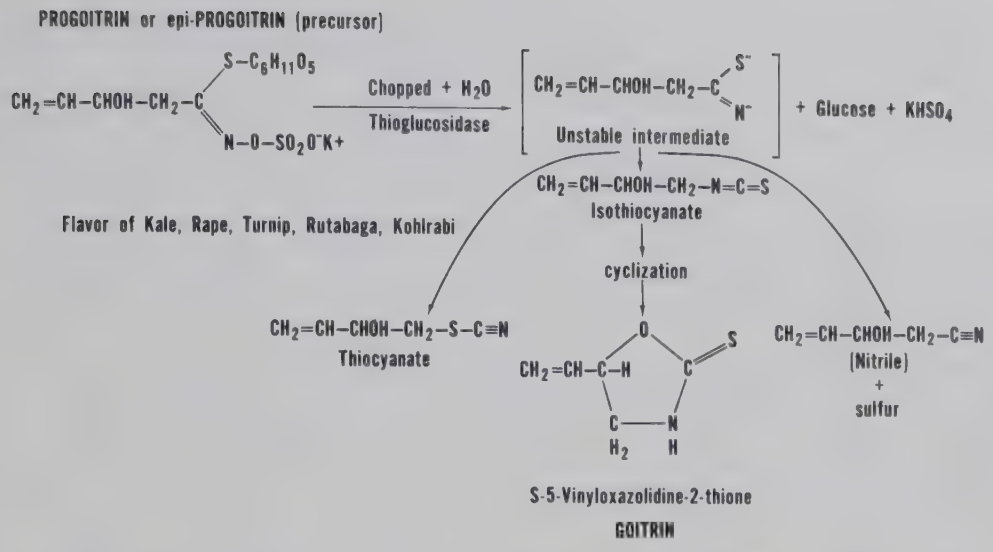


Fig. 4.–The enzymatic hydrolysis of progoitrin-a thioglucoside-to goitrin.

in healing peptic ulcers. An experimental preparation MK-72 has been prepared by Merck and Company. Steigmann and Shulman (1952) and Strehler and Hunziker (1954) have devoted further study to this antiulcerfactor. McRorie et al. (1954) isolated a heat labile analogue of methionine from cabbage juice that parallels the occurrence of vitamin U. Petering et al. (1948) also reported that cabbage contains antistiffness factors. Fujiwara et al. (1972) and Itokawa et al. (1973) shoed that *Brassicaceous* vegetables are one of the largest sources of sulfur-containing amino acids. Among these amino acids, S-methylcysteine sulfoxide, a nonessential amino acid, has a potent anti-hypercholesterolemic effect on experimental hypercholesterolemia of laboratory rats. It may be concluded that there is a possibility of S-menthylcysteine sulfoxide to retard cholesterol-related diseases.

Calloway and Munson (1961) found a substance in cabbage and broccoli that reduces the radio-sensitivity of guinea pigs when exposed to ionizing radiations. From histological and hematological studies, they concluded that cabbage and broccoli in the guinea pig diet resulted in improved nutrition and larger livers with a higher storage of vitamin A and mineral and fat content. Calloway et al. (1963) further stated that these diets supplemented with broccoli, which has a high content of β-carotene, when fed for two weeks before irradiation with X-raditation or gamma radiation, consistently and significantly reduced mortality.

Levine et al. (1932) indicated that nutritional anemia was controlled by consumption of broccoli and turnip greens and concluded that these vegetables are excellent sources of readily available iron and copper for the formation of hemoglobin. Pederson and Fisher (1944), Foter (1940), and Foter and Golick (1938) showed that allyl isothiocyanate and phenyl ethyl isothiocyanate components of crucifers have bactericidal properties. Pederson and Albury (1969) have noted that sauerkraut and its juice are laxative. Nabb and O'Dell (1964) reported that fresh and dehydrated cabbage increased *Salmonella* resistance in the guinea pig. This unidentified protective factor was water insoluble. Lakhanpl et al. (1966) and Singh et al. (1968) found that cabbage and broccoli stimulated the growth rate of guinea pigs and protected them against *Salmonellosis*. The growth stimulating effect was destroyed by drying, but the *Salmonellosis*-resistant factor was still present.

STORAGE CONSIDERATIONS

Changes in the composition of raw vegetables which may decrease their

nutritive value can occur after harvest during transportation holding, handling and subsequent storage. After they are harvested, fruits and vegetables are still physiologically active. Enzymatic and respiratory processes may bring about profound changes, unless controlled. In general, the lower the temperature at which a commodity is stored, the less likely it is to develop abnormalities with resultant loss of quality and of nutritive value. The extent of these losses depends upon the type of vegetable, its anatomical and morphological structure, rate of respiration, the content of respiratory substrates such as sugar and organic acid and external conditions.

Horticulturists, post-harvest physiologists, nutritionists, and food technologists must better understand the behavior of produce during its growth. They should know how it changes in composition as it develops, matures, and is stored before consumption. Then they can predict nutritional alterations during handling, preparation, and subsequent to storage.

Raw material handling

Leafy vegetables may be tumbled in a drum-type cleaner to dislodge sand and soil particles, but these mechanical cleaning operations can bruise or crush delicate tissues and increase respiration and oxidation of ascorbic acids. Unfortunately, literature on changes in the composition of vegetables brought about during washing and trimming for commercial processing is extremely limited. The losses that occur during such processes depend upon the crop, cultivar, maturity, freshness, and season. When leafy vegetables are trimmed, the nutrient losses generally exceed the weight losses; the nutrients tend to occur in their highest concentrations in the outer leaves.Loss of moisture from leafy vegetables by evapotranspiration occurs during holding and transporting. Washing in cold water tends to quickly rehydrate such produce.The meager published data suggest that washing can remove an appreciable amount of water-soluble nutrients from leafy vegetables.

Salad preparations and holding

Munsell et al. (1949) reported losses of 52 % or 19 % of the reduced ascorbic acid content when cabbage was minced or shredded, but losses in total ascorbic acid were only 3 % and 6 % respectively. No further losses in total or reduced ascorbic acid occurred when shredded cabbage was held for 22 hours or minced cabbage for 3 hours. However, minced cabbage showed further losses when held 22 hours. Wilcox and Neilson (1947) found that the greatest loss (10 %) of ascorbic acid occurred during the shredding or chopping of cabbage. Whether it was added with or without salad dressing, there was a slight loss. However, the addition of salad dressing increased the dehydroascorbic acid two-fold in shredded cabbage and three-fold in chopped cabbage. Wood et al. (1946) reported losses of 9 % to 15 % in ascorbic acid, 65 % in thiamine and 0 % to 3 % in riboflavin in diced cabbage with no appreciable further loss when held at room temperature for 2 hours. Francis (1960) reported that the discoloration of coleslaw was due to oxygen in the container. Tightly closed 300 MSAD cellophane of 1-mil polyethylene bags when stored bellow 50° F effectively preserved the color. The treatment of coleslaw with oxytetracycline delayed the onset of fermentation and deterioration.

Refrigeration

Haller (1947) noted that roots of turnips when trimmed, washed, and waxed stored longer and had better quality. Washing turnips improved appearance and reduced decay. Waxing reduced weight losses and produced a glossy appearance.

Zepplin and Elvehjem (1944) reported that the ascorbic acid in broccoli was destroyed rapidly at room temperature but it was significantly retained under refrigeration. Other factors such as relative humidity, temperature, exposure to air, and the physical condition of the broccoli were also important in the preservation of ascorbic acid. Ezell and Wilcox (1959) found that the wilting of cabbage, cauliflower, kale, collard and turnip greens appreciably reduced the ascorbic acid contents. Hence, control of temperature and relative humidity under refrigeration conditions is essential. They also reported in 1962 that temperature and humidity are primary factors in preserving carotene in kale, collard, and turnip greens. Wilting resulted in a rapid loss of carotene. Eheart and Odland (1972) showed that when fresh broccoli and green beans were stored at 36° F for seven days, broccoli lost no ascorbic acid but green beans lost 88 %. This difference may be due to the presence of the sulfhydryl group in the broccoli. Fager et al. (1949) found that the destruction of folic acid of fresh leafy vegetables can be reduced by stroring them under refrigeration. Olson et al. (1947) reported that the folic acid content of broccoli, cauliflower, kohlrabi, radishes, and cabbage was retained better when stored under the refrigeration temperatures. The losses were large when these vegetables were stored at room temperature.

Lyons and Rappaport (1959) reported that the respiration rate of Brussels sprouts was lower (nearly one-fifth) at refrigeration (32° F) than at room temperature (68° F) and satisfactory organoleptic quality was maintained for 72 days at 32° F. At higher temperatures (41°, 50°, 59°, and 68° F), yellowng, discoloration of stem ends, and decay occurred. Stewart and Barger (1963) noted that moistening cauliflower before vacuum cooling improved cooking quality and significantly maintained moisture content in the head. Ezcell and Wilcox (1959 and 1962) found that wilted kale, collard, turnip greens, and cabbage contained less than normal ascorbic acid and carotene. Conditions favorable to wilting, such as high temperature and low relative humidity, hastened the destruction of both ascorbic acid and carotene.

Senescence inhibitors and growth regulators. Green vegetables deteriorate steadily and rapidly after harvest (Zink, 1961). Protein content declines and chlorophyll content decreases; consequently, the loss of quality is inevitable. Kinetin-like chemicals delay senescence of green vegetables and reduce quality loss (Hruschka and Kaufman, 1949). The synthetic analogues of cytokinins have been shown to delay chlorophyll degradation and senescence of leafy vegetables. Dedolph et al.(1963) and MacLean et al. (1963) investigated that pre- and postharvest applications of N^6-benzyladenine reduced the respiration rate and extended the storage life of harvested broccoli. Salunkhe et al. (1962) found that the refrigerated life of cauliflower, radishes, and cabbage was significantly increased by a mild postharvest dip with N^6-benzuladenine and theorized that during the senescence of these vegetables, the primary step in degradation of the soluble ribonucleic acid involves the loss of the end group adenine. A treatment with N^6-benzyladenine, therefore, should provide the necessary adenine and restore the soluble ribonucleic acid molecule. Proteins and amino acids would thus be maintained and the treated produce would stay fresh longer. El-Mansy et al. (1967) confirmed the aforestated effectiveness for delaying senescence.

Isbell (1948) reported that sprouting was delayed when kohlrabi and turnip roots were treated and stored with Barsprout (a dust containing methyl ester of naphthalene acetic acid 2.2 %) and sprout inhibitor (a dust containing methyl-l-napthaleneacetate 2.2 %). Marth (1952) showed that several growth

regulators, 2-4-D and 2-4-5-D, have a pronounced retarding effect on the loss of the green color of broccoli and on cauliflower leaf abscission when applied in the field before or after harvest. However, more concrete information is needed on the toxicity of phenoxy compounds as well as senescence inhibitors to animals.

Microbe inhibitors. Since antibiotics and fungicides are not sterilizing agents, they may inhibit the growth of certain microorganisms on vegetables that are grown in or close to the soil and contain a wide range of both fungi and bacteria. Antibiotics and fungicides of broad spectra are desirable. The nutritional role of antibiotics and fungicides in vegetables at this time appears to be that of increasing the supply of high-quality perishables for a longer time by decreasing their deterioration. Vitamin K and its several analogues, especially K_3 and K_5, have markedly inhibited a number of microorganisms and would add to the nutritive value of vegetables.

Controlled- and modified-atmosphere storage. Lebermann et al. (1968) found that in the refrigerated life of broccoli, chlorophyll retention and pH increased progressively with the increase of CO_2 and decrease of O_2. Isenberg and Sayles (1969) reported that the most effective gas combination for storage of Danish cabbage was 2.5 % of O_2 and 5 % of CO_2, with N_2 making up the difference. With this combination, the weight loss due to respiration was reduced and the cabbage required less trimming. They also noted that with the atmosphere of low O_2 (2.5 % to 5 %) and high CO_2 (2.5 % to 5 %) the cabbage retained its green color, succulence, and firmness longer. Lieberman and Hardenburg (1954) showed that when modified atmospheres containing 10, 5, 2.5, or 1 % oxygen were flushed over broccoli at 75° F for 3 days, respiration was significantly reduced and yellowing of broccoli was completely inhibited. Isenberg (1973) sprayed cabbage plants one month before harvest with maleic hydrazide, Alar, CCC, N^6-benzyladenine, and Gibberellins subsequently stored them for 7.5 months at 32° F and 96 % to 98 % R. H. at 5 % CO_2 and 1 % O_2. Some chemical treatments in combination with the controlled atmosphere reduced weight and trimming losses. In addition, the controlled atmosphere reduced weight and trimming losses. In addition, the controlled atmosphere reduced 'pepper spot' diesease by reducing the rate of senescence. Controlled atmosphere consisting of 2.5 % CO_2 and 2.5 % O_2 at 32° F with a pre-storage treatment o senescence- and microbe-inhibiting chemicals, N^6-benzyladenine and mycostatin, nearly doubled the refrigerated life of polyethylene-packaged coleslaw and cauliflower florets (Salunkhe, 1973),

Radurization. The possibility of increasing the refrigerated life of fresh produce by ionizing raditon is of considerable interest to groups both in and out of the food industry, despite some setbacks. An attractive feature of the process, recognized many years ago, is the negligible temperature rise even at doses that afford complete sterilization. However, radurization (pasteurization with radiation) is not without its undesirable effects; but some progress has been made in eliminating these drawbacks. The promising area including sprout inhibition in the case of potatoes and onions, can be effectively accomplished with doses ranging from 10 to 15 kilorads, and also the retardation of ripening and microbial growth can be successfully controlled in strawberries, sweet cherries, oranges, and mushrooms with doses of about 0.25 to 0.50 megarad. In general, the success of radurization of perishable fruits and vegetables will depend upon the selection of cultivar and maturity of the commodity, semipermeable packaging material, pre-irradiation chemical treatment, and storage temperature. Ascorbic acid is the most radio-sensitive vitamin. At the pasteurization and sprout inihibition doses,

negligible losses of nutrients have taken place (Salunkhe, 1961).

To a great extent, the economic future of the refrigerated fresh produce industry depends upon intelligent use of chemicals-antibiotics, antifungals, senescence inhibitors-and packaging, handling, radurization, and storage procedures that will extend refrigerated shelf life and maintain quality and wholesomeness. Wider uses of pre- and postharvest treatments that will slow down respiration, delay senescence, and control microbial growth seem inevitable despite increasing legislative limitations on the use of food additives.

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Mechanised processing of "gari" from cassava commercial prospects

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ABSTRACT

Gari, a fermented food of cassava and a staple food in many countries of West Africa had till recently been produced at the village level by a crude, insanitary and cumbersome process. This Institute has developed successfully a mechanised process incorporating the same unit operations as in the village process. Recently a number of modifications were made in the pilot plant to improve its efficiency so as to commercialise production. This paper presents in detail the modifications made and also presents enough data to show that the mechanised process can be a feasible commercial operation.

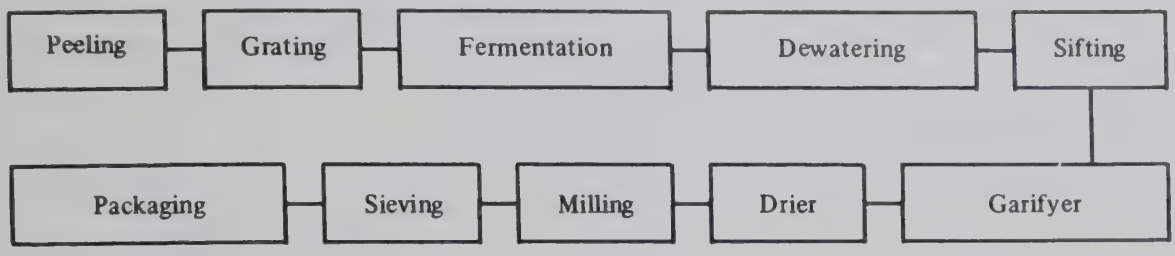
INTRODUCTION

Gari is a major staple food of the West African dietary. In some communities along the coast of West Africa it is estimated that gari contributes up to 60 per cent of the total calorie intake (Jones, 1959). Nigeria grows about 4 million tons of cassava worth about United States $84 Million, every year almost all of which is converted into "gari". The basic operations involved in the production of gari are (a) peeling, (b) grating of the cassava meat into mash, (c) allowing the mash to undergo a natural fermentation, (d) dewatering of the mash, (e) gelatinisation of the starch in the mash, (f) drying (g) packaging. In the village process all these operations are carried out in a crude and insanitary manne posing a health hazard to the consumer. The product has also a short shelf life; its high moisture content rendering it prone to mold attack. In the mechanised process developed by the Federal Institute of Industrial Research (FIIR), however most of the operations can be carried out untouched by hand and the various paramaters controlled to yield a standard product with a shelf life of more than a year.

Akinrele et al (1971) have given the industrial specifications for the mechanised processing of cassava into gari in detail. This paper gives the results of the working of the pilot plant under commercial conditions and the improvements made in the plant. Further on the basis of these results a projection has been made on the commercial suitability of a plant with an installed capacity of 10 ton sin 24 hours.

EXPERIMENTAL

The flow sheet of the pilot plant as used for this investigation is given below:



The cassava roots after weighed were cut into pieces not more that 10 cms long and then transferred into the mechanical peeler. The peeler consist of a drum coated with abrasive and rotates eccentrically on a shaft. Each charge consisted of 58 kgs. (150 lbs) of cassava and to this was added 68 l (15 gallons) of water. The peeler was switched on and the drum rotated for 3 minutes. After this the material was discharged into wire mash trays, washed with water to remove all the debris and then trimmed by hand with paring knives to remove any adhering peel. Records were kept of the operation. Peeling of the whole cassava was also done by hand by using manual labour and results compared with machine peeling.

The trimmed cassava was then grated by using a Robert Fries' grater and then stored in both aluminium vats and fibre glass containers. They were, however, seeded with cassava liquor from an earlier dewatering operation to hasten fermetation. Once the pH reached 4.0 the mash was dewatered. Two methods were used for dewatering and the results compared. In one method about 23 kg (50 lbs) of mash were packed into nylon bags, the bags were tied up with strings at one so that no mash could escape. 10 bags wer loaded into a hydraulic press and then subjected to 100 lbs sq. inch pressure for 5 minutes by means of a ram which could move horizontally. The liquor from the mash was allowed to escape through the nylon bags into a perforated tray kept at the bottom and then collected to be used for seeding fresh mash. In the second method the mash was loaded into a basket centrifuge. The basket was lined with canvas and allowed the liquid to seep through while being centrifuged. The centrifuge could take approximately a load of 45 kgs of mash at a time and the normal time for the whole operation of a batch was ten minutes. Some batches, however, took a longer time and in extreme cases as much as 45 minutes was required. At this stage, the moisture content of the mash was between 47 to 50 %. After dewatering, the mash was fed into a mechanical sifter. The sifter consisted of a stationary sieve with a rotating shaft in the centre to which were attached blunt knives. There was a small clearance between the sieve and these blunt knives. The mash which was fed from a hopper at the top was broken up and all the fibre and large pieces were sieved out and collected separately. The mash which went through the sieve was fed by means of an elevator into a "garifyer". In the garifyer which consisted of a rotating drum with baffles (for complete description see Akinrele et al 1971) the mash was subjected to a high temperature (inlet 300-320°C; and outlet 250°-280°C) for about 15 to 20 minutes. This gelatinised the starch and reduced the moisture to about 40 %. From the garifyer the gelatinised mash was fed into a louvre dryer with an inlet temperature of 200° C and outlet temperature of 145° C and the moisture content brought to about 8 % in 15 to 20 minutes. The hot, agglomerated product was allowed to cool and then ground in a disc mill. The graded and sieved gari was then packaged as "gari" which consisted of the fines into 5 lb polythens bags and then distributed for sale.

RESULTS AND DISCUSSION

In Table 1 is presented the overall time taken by the machine for peeling the cassava as compared to that done by hand. It is seen that the time (calculated as man hours) taken by the machine is about half that taken when the peeling is done by hand. As the size of the operations increase, the number of women needed for hand peeling would also increase so that at a level of about 30 tons in 24 hours as much as 215 workers would be needed. This would mean that in each shift there would be about 72 workers for peeling alone. We feel that any operation using more than 70 workers in a shift for peeling would be difficult to manage especially in view

TABLE 1

Comparison between machine peeling and hand peeling of cassava

Run No.	Time taken by machine and further hand trimming for 1000 kg in man hours*	Time taken for hand peeling of 1000 kg of cassava in man hours*
1.	14.5	30.2
2.	14.0	31.5
3.	15.2	30.8
4.	14.7	31.7
5.	14.5	30.7
6.	15.3	32.6
7.	15.6	36.3
8.	15.5	30.8
9.	16.0	31.2
10.	15.3	30.5
11.	14.6	29.8
12.	15.5	31.2

*** Each run is an average calculated from the running of a plant for seven days.**

of the further supervisory staff that would be needed to weigh and keep track of the whole operation. It would thus be economical and more efficient to use the peeling machine once the intake of cassava is more than 30 tons in 24 hours.

Studies showed that storing the mash in reinfored fibre glass plastic containers did not produce any off-flavour in the final product. Further, it was found difficult to transfer manually all the grated mash into the containers. It is suggested that some type of mono pump be devised to pump the mash from the grater into the fibre glass containers.

Table 2 shows the results obtained for dewatering the mash by centrifuging and by using the hydraulic press. Results obtained definitely show the superiority of the centrifuge over the press for dewatering. Further the centrifuge could be fitted into an automated process much better than a hydraulic press. However workers at a factory in Gambia claim that the press is more efficient than the centrifuge. More work would have to be done to determine which is the better method. If the centrifugal method is better, bigger centrifuges with higher capacities would have to be designed to handle large quantities of mash.

The mechanical sifter used during the operation was successful in removing the fibres and the larger particles from the mash and thus improved the efficiency of the later operations of garifying, drying, milling and sieving. The average percentage of fibre and mash removed during this operation was not more than 1 per cent of the gari produced. Once the mechanical sifter was used the final rejects were less than 0.2 per cent. When the mechanical sifter was not used the percentage of rejects from the final product used to be in the region of 2.0 per cent.

The mash was lifted into the garifyer either manually or by means of an elevator which had been improvised. In the light of the difficulties encountered a bucket elevator would be the answer to the problem. Such an elevator has been designed and is expected to be put into operation in commercial plants being erected at present in Nigeria.

A small cyclone was introduced before the hot air from the louvre dryer passed into the atmosphere to collect any fines which would tend to pollute the atmosphere.

TABLE 2

Comparison between dewatering by two methods

Run No.	Time taken to dewater 100 kg of cassava mash by centrifuge in man hour*	Time taken to dewater 100 kg of cassava mash by hydraulic press in man hours*
1.	0.45	0.68
2.	0.43	0.72
3.	0.48	0.80
4.	0.51	0.88
5.	0.75	0.89
6.	0.52	0.87
7.	0.56	0.83
8.	0.47	0.79
9.	0.48	0.88
10.	0.52	0.89
11.	0.53	0.79
12.	0.78	0.80

*** Each run is calculated on the basis of one day's (24 hours) working.**

TABLE 3

Recommended standards for mechanised processing of cassava into gari

Item	Standard	Remarks
Peeling.	23±2 %	Calculated on weight of cassava roots
pH after final fermentation.	4.0±0.15	
Acidity after final fermentation.	0.85 %	Expressed as lactic acid.
Moisture content after dewatering.	49±2 %	
Moisture content of final product.	8±0.5 %	
Fuel consumption.	55 gallons of diesel oil	On the basis of 1 ton of gari produced.
Final yield.	24±1 %	Based on the weight of cassava roots.

TABLE 4

Projected figures for the working of a 10 ton a day gari plant

Item	Actual figures for 2.5 ton a day Pilot Plant	Projected figures for 10 ton a day Plant
Cassava roots.	10 tons	40 tons
Fuel as Diesel oil.	137.5 gallons	550 gallons
Labour Needed.	25 men	30 men
Final yield of gari.	1 ton	2.5 tons
Profit before depreciation.	30 %	40 %

Before the introduction of this device, the area around the plant was strewn with gari fines which after a few days produced an obnoxious smell. After the cyclone was introduced there was hardly any fines comming out of the chimney.

Table 3 shows the standard loss of moisture and other constituents at the end of each operation and the standard yield in the light of the results obtained after continuous working for a period of six months. It is seen that a standard yield of 25 % of the raw cassava could be expected as gari.

Table 4 shows the extrapolation from our pilot plant (capacity 3.5 tons) studies to the working of a 10 ton a day plant. These studies show that the working of a 10 ton a day plant is commercially feasible. As plants with capacities of 10 tons a day are being put into operation shortly in Nigeria, it is hoped that figures would be available from these plants on actual run basis for comparison with our projected figures.

CONCLUSION

A commercial plant capable of producing 10 tons of gari a day should be feasible. What is needed is a regular supply of cassava throughout the year. This would be possible if a cassava plantation were attached to the plant.

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Characteristics of high protein spaghettis supplemented with soy proteins

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ABSTRACT

Spaghettis products with high protein content (20 to 50 %, 12 % moisture basis) have been processed in a pilot plant from durum wheat semolina supplemented with soy proteins. Brown index, cooking loss and firmness of spaghetti increase generally with the addition of soy protein. According to the overall technological quality of spaghettis, as confirmed by a panel of degustation, "Promine", a soy isolate, is the more promising product when compared to other soy proteins. In every case, control spaghetti from durum wheat semolina have a better color and a lower tenderness after cooking.

INTRODUCTION

Cereals are poor in lipids and contain only 8 to 15 % proteins; they are generally considered as a source of energy for human nutrition, because of their high starch content. In fact cereals constitute an important source of protein, by giving about 50 % of the human food proteins. These proteins are of low biological value, because of their poor essential amino-acids content, such as lysine.

Paste products are universally known. Besides, they are easily stored and can be processed from many kinds of cereals. Accordingly, it is of special interest to improve their nutritive value (Clausi, 1971). But nutritionaly improved products can be successfully obtained only if they have organoleptic qualities at least similar to those of there substitutes. Soy protein supplemented paste have been manufactured and their flavor, color and cooking quality have been compared with paste made from durum wheat semolina.

MATERIALS AND METHODS

1- Sample processing.

1-1 Blend of durum wheat semolina and soy product.

Commercial durum wheat semolina has been used; soy products are described in Table 1.

TABLE 1

Processing of high protein spaghettis supplemented with soy proteins. Characteristics of the soy products and durm wheat semolina

	Protein content (12 % M.B.)	Observations
Soy products - Trade name.		
Isolates Promine D.	78.4	Gelling Agent
Promine F.	82.0	Non Gelling Agent
Promine R.	84.6	Isoelectric Protein
Concentrates Promosoy 100.	63.4	Finely Ground Concentrate
Promosoy 20/60.	63.4	Coarse Concentrate
Flours Soyafluff 200 W.	50.2	Defatted Flour
Soyafluff 200 T.	50.2	Defatted and Toasted Flour
Hizyme 280.	49.7	Defatted Flour-Active Enzyme
Durum Wheat Semolina.	12.2	

TABLE 2

Processing of high protein spaghettis supplemented with soy proteins. Water content before extrusion (Percentage, 12 % M.B.)

Protein content of the spaghetti (12 % M.B.)	20 %	28 %	40 %	50 %
Blend with:				
Promine D.	30.7	32.9	39.7	47.7
Promine F.	34.7	36.8	41.3	47.6
Promine R.	34.7	36.8	41.3	–
Promosoy 100.	30.7	41.2	–	–
Promosoy 20/60.	–	41.2	–	–
Soyafluff 200 W.	29.7	28.0	–	–
Soyafluff 200 T.	–	28.0	–	–
Hizyme 280.	27.8	25.0	–	–
Control of durum wheat semolina.	31.4			

Several blends have been made in order to obtain spaghetti with protein contents of 20; 28, 40 and 50 p. 100 (12 % moisture basis) and, in any case, with at last 50 p. 100 of durum wheat semolina.

1-2 Hydratation

The blend was first hydrated and mixed by placing 1 kg in a 4 bladed single shaft trough mixer (48 r. p. m.) and adding the water slowly (Table 2).

Mixing was carried on for 15 minutes. The resulted product was crumbly and fairly free-flowing. It was then transfered in the mixing section of the extruder DEMACO Press. This section consisted of a trough with two shafts each with 5 blades revolving at 100 r. p. m. The second stage of mixing was carried on for 5 minutes.

1-3 Extrusion.

From the mixing section of the extruder, the mixture was propelled to a water jacketed (50° C)

worm extruder. During extrusion, a partial vacuum of 0,7 bar was applied. The spaghettis are processed with a flow rate of 6 kg/h.

1-4 Drying.

The spaghettis were dried in a VOTSCH VKI 01/250 cabinet with a programmed temperature and hygrometry. Drying cycle is shown in Figure 1.

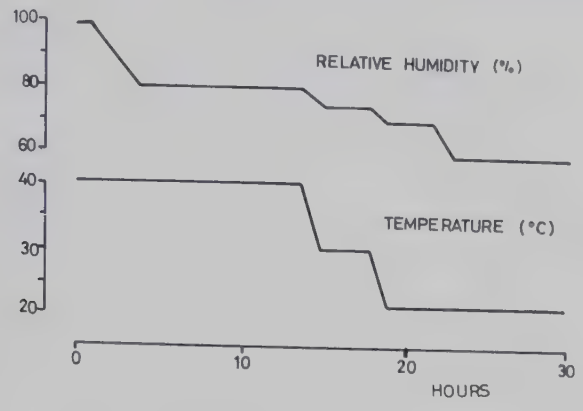


Fig. 1.–Drying cycle of the spaghettis.

2- Evaluation of samples quality.

2-1 Color.

The spaghetti are ground. Color is measured with the disc-method of ALAUSE and FEILLET (1970). Two index are obtained:

— Brown index (B. I.) = 100 x O D at 550 nm

— Yellow index (Y. I.) = (100 x 0 D at 480 nm) - B. I.

A well colored spaghetti is characterized by a low Brown Index and a high Yellow Index.

2-2 Cooking tests.

10 g. sphaghetti are cooked 10 and 20 minutes in 100 ml water. Temperature is keept at 98° C in a thermostated water bath.

Paste are weighed after draining. Swelling is the quantity of water absorbed by 100 g. crude spaghetti during cooking.

Firmness of cooked paste is measured by the force necessary to extrude 10 g. of sample through a 2 mm hole in a plate. Results are expressed in kg.

Cooking water is evaporated for 15 hours at 105° C and residue is weighed. Residue is given in g. for 10 g. crude paste as an index of cooking loss.

2-3 Degustation.

Bitterness of the spaghettis has been test by test panel.

RESULTS AND DISCUSSION

Results are summarized in Tables 3, 4, 5 and 6.

First, we shall show the influence of various soy products on the quality of the spaghettis, when we aim at obtaining a 28 % protein content in spaghettis. Them we shall study the quality evolution of the pastes, when incorporating increasing dose of soy isolated proteins.

1- Influence of the introduction of vvarious soy products on the quality of spaghettis with 28 % protein content.

1-1 Color.

In any case, except for concentrated protein "Promosoy 20/60", brown index is increased when compared to control. This increase reaches 78 p. 100 for "Soyafluff 200 T" flour and can be attributed to toasting of this product.

The yellow index varies with the different products. It is the lowest with

TABLE 3

Color of high protein spaghettis supplemented with soy proteins. (B I = Brown Index, Y I = Yellow Index)

Protein content of the spaghetti (12 % M.B.)	20 %		28 %		40 %		50 %	
Color index	BI	YI	BI	YI	BI	YI	BI	YI
Spaghetti supplemented with:								
Promine D.	17.2	21.5	19.7	21.2	22.3	20.0	23.8	20.0
Promine F.	17.7	16.4	18.5	16.4	20.0	17.2	23.2	18.7
Promine R.	17.7	16.0	20.1	15.0	21.3	16.0	–	–
Promosoy 100.	18.9	19.0	20.9	25.5	–	–	–	–
Promosoy 20/60.	–	–	16.6	13.7	–	–	–	–
Soyafluff 200 W.	19.3	17.0	21.5	21.5	–	–	–	–
Soyafluff 200 T.	–	–	30.2	20.0	–	–	–	–
Hizyme 280.	19.7	12.1	25.3	12.6	–	–	–	–
Spaghetti control.	17.0	18.2						

TABLE 4

Cooking test of high spaghettis supplemented with soy proteins. Swelling of the cooked spaghettis (g of absorbed water/100 g of crude spaghetti)

Protein content of the spaghetti (12 % M.B.)	20 %		28 %		40 %		50 %	
Cooking time (mn)	10	20	10	20	10	20	10	20
Spaghetti supplemented with:								
Promine D.	131	177	136	178	126	179	140	198
Promine F.	134	185	124	167	128	178	134	196
Promine R.	142	195	131	173	98	135	–	–
Promosoy 100.	130	182	132	186	–	–	–	–
Promosoy 20/60.	–	–	129	208	–	–	–	–
Soyafluff 200 W.	136	196	132	195	–	–	–	–
Soyafluff 200 T.	–	–	134	192	–	–	–	–
Hizyme 280.	130	173	132	167	–	–	–	–
Spaghetti control.	154	219						

"Hizyme 280": this result is supposed to be due to the oxidation of carotenoid pigments by the active enzymes of the product.

Finally, with a product of 28 % protein content, only the mixtures with "Promine" or "Promosoy 100" are acceptable from the point of view of color.

1-2 Cooking test.

Each product has a lower swelling and a higher firmness than the control. Cooking loss are generally higher except with "Promine" D. F and R.

1-3 Degustation.

Bitterness is negligible only for the spaghettis supplemented with "Promine".

From this standpoint, only the isolates "Promine" would be susceptible to be used for enriching spaghettis in soy proteins.

2- Effects of the incorporation of increasing level of isolated protein "Promine" D. F and R.

2-1 Color.

Brown color is positively correlated with protein content. But the choice of

TABLE 5

Cooking test of high protein spaghettis supplemented with soy proteins. Firmness of the cooked spaghettis (Kg)

Protein content of the spaghetti (12 % M.B.)	20		28		40		50	
Cooking time (mn)	10	20	10	20	10	20	10	20
Spaghetti supplemented with:								
Promine D.	7.8	4.0	9.6	4.3	10.7	5.9	10.1	7.3
Promine F.	6.0	2.9	7.8	3.7	9.4	3.8	14.1	6.9
Promine R.	5.1	2.4	5.5	2.8	11.3	11.3	–	–
Promosoy 100.	8.3	4.5	7.7	3.9	–	–	–	–
Promosoy 20/60.	–	–	7.2	3.6	–	–	–	–
Soyafluff 200 W.	7.9	4.0	7.3	4.2	–	–	–	–
Soyafluff 200 T.	–	–	6.0	3.9	–	–	–	–
Hizyme 280.	7.0	3.7	7.3	3.8	–	–	–	–
Spaghetti control.	5.2	2.4						

TABLE 6

Cooking test of high protein spaghettis supplemented with soy proteins. Cooking loss (g of residue/10 g of crude spaghetti)

Protein content of the spaghetti (12 % M.B.)	20		28		40		50	
Cooking time (mn)	10	20	10	20	10	20	10	20
Spaghetti supplemented with:								
Promine D.	0.55	0.61	0.50	0.65	0.53	0.71	0.46	0.78
Promine F.	0.60	0.67	0.60	0.68	0.61	0.65	0.59	0.81
Promine R.	0.55	0.63	0.65	0.69	0.60	0.65	–	–
Promosoy 100.	0.43	0.70	0.50	0.86	–	–	–	–
Promosoy 20/60.	–	–	0.65	0.83	–	–	–	–
Soyafluff 200 W.	0.39	0.86	0.77	1.11	–	–	–	–
Soyafluff 200 T.	–	–	0.86	1.12	–	–	–	–
Hizyme 280.	0.67	0.75	0.82	0.88	–	–	–	–
Spaghetti control.	0.52	0.67						

"Promine" F limits to some extent the indesirable increase of the brownness.

The yellow index does not increase very much with the protein content increase.

It is yet possible to obtain fairly reasonable color using high protein contents.

2-2 Cooking test.

As already seen, the swelling of spaghettis enriched with "Promine" is lower than that of spaghetti control. It is necessary to make a distinction between "Promine" D and F and "Promine" R. When using "Promine" D and F, wich are sodium proteinates, respectively gelling and not gelling, swelling increases with the protein content, but keeps lower than that of the control. On the other hand, when using the isoelectric protein "Promine" R swelling decreases with protein content, spaghettis become more "compact". For the three kinds of isolates "Promine" D, F and R, firmness increases with protein content, to a lower extent with "Promine" F. However, it is very high compared with control.

Cooking losses are comparable for "Promine" supplemented spaghettis and control spaghettis.

The cooking behaviour is the best for "Promine" R supplemented spaghettis untill 28 % whole protein content. Beyond this content, the best cooking tests are obtained with "Promine" F supplemented spaghettis.

2-3 Degustation

The bitterness is negligible until 28 % of protein content and low after this concentration.

CONCLUSION

The incorporation of soy proteins in spaghettis is a valid way to improve quality and quantity of the proteins of the products. (See Annex). We did not meet with important difficulties when processing the products: supplemented paste can be manufactured with existing apparatus. However, we must report that a greater quantity of water is necessary and must be appreciate with preliminary trials; therefore drying is more expensive with regard to the energy.

As far as sensory evaluation is concerned, the bitterness due to soy proteins is the main deficiency regarding acceptability by consumers. For that reason at high level,only isolates as "Promine" whose bitterness is very weak and color light could be used. But in any case, soy proteins supplemented spaghettis have a overall quality inferior to that of durum wheat semolina spaghettis.

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ANNEX

Composition in essential amino-acids of spaghettis supplemented with soy proteins.

This composition has been computed from data of FAO (1970, 1973). It is not an experimental study; the essential amino-acids contents and the chemical score* of the spaghettis are summarized in Table A 1. They have only an indicative value.

TABLE A 1

Essential aminoacids of the spaghettis supplemented with soy proteins (g/16 g proteins)

	Protein content (12 % M.B.)	Isoleucine	Leucine	Lysine	Methionine + Cystine	Phenylalanine + Tyrosine	Threonine	Tryptophane	Valine	Chemical score (%)	Limiting aminoacid
Standard (FAO, 1973).		4.0	7.0	5.5	3.5	6.0	4.0	1.0	5.0		
Semolina.	12.2	3.6	7.0	2.1	3.9	7.1	2.7	1.1	4.1	38	Lysine
Spaghetti with:											
Promine D.	20	3.8	6.9	3.4	3.1	7.4	2.9	1.0	4.0	62	Lysine
Promine D.	28	4.2	7.5	3.6	2.5	8.2	3.0	1.1	4.8	65	Lysine
Promine D.	40	4.4	7.6	4.8	2.5	8.4	3.4	1.1	5.1	71	S
Promine D.	50	4.5	7.7	5.2	2.4	8.5	3.5	1.1	4.4	68	S
Spaghetti With:											
Promine F.	20	3.9	7.3	3.5	3.3	8.0	3.0	1.1	4.2	64	Lysine
Promine F.	28	4.1	7.5	4.4	2.9	8.0	3.2	1.1	4.4	80	Lysine
Promine F.	40	4.2	7.6	5.1	2.6	8.3	3.3	1.2	4.5	74	S
Promine F.	50	4.4	7.7	5.5	2.4	8.5	3.4	1.2	4.5	68	S
Spaghetti with:											
Promine R.	20	3.9	7.2	3.4	3.3	7.7	3.0	1.0	4.2	62	Lysine
Promine R.	28	4.2	7.4	4.2	2.8	8.0	3.2	1.1	4.3	76	Lysine
Promine R.	40	4.4	7.6	4.8	2.5	8.4	3.4	1.1	4.4	71	S
Spaghetti with:											
Promosoy.	20	4.1	7.4	4.0	3.4	7.9	3.4	1.3	4.4	73	Lysine
Promosoy.	28	4.3	7.5	4.9	3.1	8.3	3.8	1.4	4.6	88	S
Spaghetti with:											
Soyafluff.	20	4.0	7.3	4.2	3.4	7.9	3.3	1.2	4.4	76	Lysine
Soyafluff.	28	4.2	7.5	5.1	3.2	8.2	3.6	1.3	4.6	90	Threonine
Spaghetti with:											
Hizyme 280.	20	3.9	7.3	3.7	3.5	7.7	3.2	1.2	4.4	67	Lysine
Hizyme 280.	28	4.1	7.4	4.8	3.2	8.1	3.5	1.3	4.5	87	Lysine

*Chemical score: the content of each essential amino-acid in a food is expressed as a percentage of the content of the same amino-acid in the same quantity of the reference pattern of amino-acids. The amino-acid showing the lowest percentage is called the limiting amino-acid and this percentage is the chemical score.

In any case, the fact of supplementing durum wheat semolina with soy proteins increase the chemical score of pasta. Generally the most favorable chemical score is obtained when choosing a protein spaghetti content of 28 %. At that content the best results are obtained with soy flours and concentrates; but these products are at the limit because of taste and color.

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Development of technological procedures for production of new spice concentrates and extracts from paprika

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ABSTRACT

Technological procedures for the production of new spice concentrates and extracts from paprika where the production is based on the modified serum procedure, patented by Sulc and Ćirić (1965) have been evaluated and tested even under industrial conditions.

Quality of the obtained products is shown in Table 1 for serum concentrates and pulpy paprika concentrates and in Table 2 for the aqueous and oily ones.

High-quality of the obtained products involves their usage as a spice and additive in all of the cases where the ground red pepper is used today, i.e. for the aromatization and colouring of various food products.

INTRODUCTION

The development of technology and technics of juices concentrating in the last twenty years, have lead to the construction of plants which involve the operation of juice concentrating in a short period and at relatively low temperatures, (Siegrist, 1960, Pilnik et al., 1960). The further advancement in concentrated juices production was carried out by aroma recovery plants. The result was the reduction of flavour substances during the concentrating process (Milleville et al., 1944). Among the mentioned plants, those working under vacuum are favoured due to the higher degree of juice and flavouring substances evaporation. The possibility of oxidative changes on dearomatized juice and on concentrated.aroma which is obtained, is avoidable (Sulc, 1963, Bomben, 1966, Sulc, 1970).

Modern plants produce clear concentrates of high quality from depectinized and filtrated juices. The attempts for mashed or pulped concentrates production from fruits and vegetables pressed thorough the screen have failed except in the case of tomato processing in well-known "tomato lines".

We have worked intensively on this problem last ten years; using the principles of "serum procedures" (Pimazzoni, 1961) we have worked out in details and patented a new modified serum procedures (Sulc and Ćirić, 1965, 1968, 1969)

which has given the possibility of producing high quality pulpy concentrates from various fruits and vegetables. This new procedures, addapted for paprika has enabled the production of new spiced concentrates and extracts from paprika, what will be discussed in this paper.

The new products can completely replace ground paprika, which has served as spice and additive in various semi-finished meat products and ready dishes and other food products. Because of old technology or production and unsuitable manner of preservation, due to complex reactions of nonenzymic browning, far reaching destruction of the colours of ground paprika caramelization and partial breadkdown of sugars, as well as destruction of vitamins, occur. Furthermore, the product is very contamined with bacteria and molds and as spice it is a cause of infection for food products it has been added to,
New technology of paprika processing in spice concentrates and extracts of high quality have been of great importance.

EXPERIMENTAL

Investigation was carried-out by the following sorts of garden paprika: Green Rotunda, California miracle, Pazardzijska kapja, Kurtovska kapja and spice sort of Horgoska sweet I, which is especially used for ground paprika production. All sorts of paprika were intensively red and in full ripeness.

After washing and selecting, the raw material was rough ground and immidiately heated in the tube heat exchanger at 85° C and cooled at 45° C. At this temperature it was treated with suitable pectolytic enzymes. The mass was heated at 80° C and pressed through the screen. The obtained mash was separated through the centrifugal decanter, which separates the serum (liquid phaze) and pulp (solid phaze).

Serum was centrifuged and concentrated in low temperature film evaporator to "serum concentrate" with cca 70 % of total solids. This product can be used as spice concentrate or mixed with pulp as "pulpy concentrate".

Pulp was mixed with the initial mash in proportion 1 : 1, based on "cut back" principle and allowed to pass the korund-mill before mixing with serum concentrate and thus obtained mixture was mixed with the aliquot part of serum concentrate. The obtained *pulpy concentrate* was deaerated, pasteurized at 85° C, hot filled into five kilogrammes tins, which were cooled and stored at +2° C till further processing.

Serum concentrate was filled into five kilogrammes tins, but it may be filled into special 50, 100, 200 kilogrammes casks, which are protected with epoxy resins. It was stored at +2° C till further processing, as well.

Waste products (seed, epidermis, green parts) were extracted by aceton, after they were pressed through the screen. After the solvent was removed from miscela, an aqueous-oily extract was obtained, which was than separated by centrifuge, giving the aqueous flavouring and oily coloured extract of paprika, the so-called oleorisine. The extract may be filled in suitable tins or in brown-glass bottles and stored at +2° C till further processing.

The quality of industrial concentrates and extracts were determined by the standard chemical methods (A. O. A. C., 1970). The total number of microorganisms was determined by Koch (Zakula et al., 1969). The colours of concentrates and extracts were determined by the modified method by Benedek (1958). Flavouring of products was determined by gas chromatography. This method is based on the criterion of total area of the separated componets (Ćirić, 1973). Identification of volatile components was determined by retention time of test substances, in aqueous flavouring extract (Ćirić, 1973). Qualitative determination of oleorizin's coloured fraction was based on thin-layer chromatography (Al_2O_3, MgO, $CaSO_4$) and spectrophotometric measurements at 250-550 nm, with methanol as solvent. Identification of carotenoids was carried out by the determination of wave-lenghts of maximum absorbtion and comparison with Vinkler et al. (1972) results. Fatty acid composition of oleorizine was determined by gas chromatography using their methyl esters (Ostrić-Matijasević, 1964). Tocopherol content was determined by Emmery-Engel method (Emmery-Engel in Paquot, 1967).

RESULTS AND DISCUSSION

Yield of the obtained products

The applied technological procedures have given the qualitative concentrates and extracts under industrial conditions, both of garden sorts of paprika as well as of spice paprika, with the satisfactory yield. Yield of the obtained products, calculated on the initial amount, varried, with the characteristics and total solids of paprika, and it was:

— Serum concentrate of paprika with 70 % of total solids ..... 8 - 12 %
— Pulpy concentrate of paprika with 50 % of total solids ..... 15 - 20 %
— Aqueous flavouring extract of paprika ..... 0,1 - 0,3 %
— Oily coloured extract of paprika (oleorizine) ..... 0,5 - 1,0 %

Quality of the obtained products

Average chemical composition, microbiological pattern, the colour and flavour of serum and pulpy concentrate compared with the ground paprika, are presented in table 1.

According to the data given in tab. 1., we can make conclusion that these concentrates of paprika posses considerably higher sugar content and vitamin C content, colouring and flavouring intensity but lower ash content, crude oil content, proteins and cellulose content then ground paprika, having thus better quality and shelf-life.

TABLE 1

The quality of serum and pulpy concentrate

Constituents (%)	Serum concentrate	Pulpy concentrate	Ground red pepper
Total solids.	60.0-72.0	50.0-52.0	94.0-96.0
Soluble solids refr.	69.0-71.0	49.0-51.0	–
Tot. invert sugar.	47.0-57.0	45.0-48.0	24.0-26.0
Dir. reduct. sugar.	45.0-55.0	40.0-46.0	22.0-24.0
Sucrose.	3.0-5.0	5.0-8.0	0.8-1.2
Titr. acidity.	1.3-2.5	1.6-2.8	1.5-1.7
Ash.	3.0-5.0	3.5- 6.0	6.0-7.0
Crude oil.	1.3-2.8	2.4-4.0	10.0-11.0
Ca-pectate.	0.3-0.7	1.0-2.0	2.0-3.0
Proteins.	3.0-4.0	4.0-5.0	14.0-15.5
Cellulose.	0.3-1.0	4.0-4.8	11.0-13.0
Vitamin C, mg %.	1200-1300	800-900	–
Tot. number of microorganisms in g sample.	150-200	150-200	100,000- -120,000,000
Colour g/kg of total solids.	6.0-7.0	8.0-9.0	2.0-4.0
Flavour intensity/total area of aromagrams in mm^2.	80,000- -90,000	110,000- -120,000	40,000- -50,000

Microbiological pattern of the obtained paprika concentrates was better (about 200 microorganisms per gramme) then that of ground paprika, where the number of microogranisms was more then 100,000.000 per gramme.

Flavouring of pulpy concentrate is much higher then that of serum concentra-

te, because the most part of flavour is in the fleshy part, which is one of the components of pulpy concentrate but not of serum concentrate. Flavouring of ground paprika is twice or three times less then flavouring of serum or pulpy concentrate, what is quite normal considering the technological procedures.

We can conclude that serum concentrate and pulpy concentrate of paprika may be used with better efficiency as spice and additive in all cases where we use ground spice paprika. The chemical composition and quality of aqueous (flavouring) and oily coloured extract of paprika are showen in table 2. *Aqueous flavouring extract* of paprika possesed proportionally low total solids (0,2-0,3 %) and extremenly high flavouring intensity expressed as a total planimetry area of chromatogram. Investigation of volatile components —flavouring and non-flavouring— of paprika have been the subject of docent Ćirić (1973) dissertation, who was found that the flavour of paprika has many components, i. e. 119 components. Flavours was rich in chemical groups as well.

TABLE 2

The quality of paprika extract

Aqueous flavouring extract:	Oily extract (oleorisin):
Volatile components:	*Colouring matters:*
49 different alcohols and estars out of which the following are identified: 12 alcohols and 15 esters;	Beta-carotene, cryptoxanthin, lutein, zeaxanthin, cryptocapsin, beta-cytaurin, capsanthindipalmitate, violaxanthin and capsorubindipalmitate.
24 volatile acids/7 of them were identified;	*Fatty acid composition of oil:*
33 phenolic compounds/2,5-xylenol is identified/;	Linoleic (74.73 %), palmitic (12,63 %), oleic (8.7 %), stearic (2,98 %), arachidic (0.32 %), palmitoleinic (0.28 %) and lauric and myristic acids only in traces.
13 carbonyl compounds (11 aldehydes and 1 ketone are identified).	Tocopherols, 80 mg %
Flavour intensity:	Oils, 98 %
Expressed as a total planimetry area of aromagrams, 19^6-10^7 mm^2	Colouring by Benedek, 11-15 g/kg dry matter.
Total solids,0.2-0.3 %	

Twelve alcohols were identified and the most frequent were: methanol, ethanol, crotyl alcohol, geraniol, nerol and so on. The most frequent among fifteen identified estars were: ethyl acetate, ethyl caproate, methyl caporate, ethyl valerat, propyl acetate, ethyl isovalert, ethyl cinnamat, isopenthyl acetate, ethyl crotonnate and so on.

Identified volatile acids were: acetic acid, isovaleric acid, capric acid, caporic acid, caprilic acid, butyric acid and isobutyric acid.

We separated 33 phenols, but only 2,5-xylenol was identified. The most frequent among 11 identified aldehydes were: heptyladehyde, propionaldehyde, butyraldehyde and salicylaldehyde. Only isoporpyl methyl ketone is identified.

Flavouring composition of paprika extract is identical with flavour of concentrate, but the quantitative relation of total and individual components is higher in extract.

Oily paprika extract - oleorisine has higher intensity of colour (11-15 g/kg determined by Benedek), high oily content (98 %) and tocopherol (80 mg/100 gr) which gives it the biological and antioxidative value.

Identification of carotenoides in coloured extract was based on Vinkler's results (Vinkler et al., 1972). These authors have used Silica gel G as adsorbent, but magnesium-aluminium oxides have also given good results. Amoung 11 coloured fractions, nine were identified.

Their absorbtion spectra enables quantitative determination of individual fractions. The highest peaks have been found for beta-cytaurin, capsanthin and capsorubin whose quantities are predominant among all other carotenoides and have given the characteristic red colour to paprika.

Gas chromatography analysis proved fatty acid composition of oily paprika extract. According to these results oily paprika extract is relatively rich in fatty acid. Among 12 recorded acids, eight were identified out of which the most frequent was linoleic acid belonging to the group of essential fatty acids. This acid gives the particular biological value of oily extract. This oil belongs to the group of semi-dry oils in regard to high content of unsaturated fatty acids /cca 84 %, of oil/, so it can be used in chemical industry, especially in the industry of dyes.

Oily extract is rich in tocopherols which are the antioxidants and vitamin substances of importance in nutrititon / alfta-tocopherol/. Content of tocopherol in oily extract have showed relatively good biological and antioxidativ value of this paprika product.

CONCLUSION

In regard to the fact that the serum and pulpy concentrates posses higher physiological values, more intensive colour and flavour and more suitable microbiological pattern then the ground spice paprika, they can be used as spice and additives in all cases where the ground spice paprika is used today. Some of our investigations have showed that serum and pulpy concentrates of paprika were technologicaly more suitable then a ground spice paprika in cream cheese and sausage production. The taste, aroma and colour of paprika are more intensive in sausage and cream cheese production.

The uncoloured extract of paprika can be used successfully for flavouring the products were only the taste and aroma of paprika are prefered /various kinds of cheese and meat products/. Coloured oil extract /oleorisine/ opposite to clasical oleorisine, which is produced from ground spice paprika, has more intensive flavour and colour whereas its use in food technology is more successful.

The developed technological procedures enable almost the complet using of paprika as a raw-material, because the waste-products may be used as feed, applicable for the nutrition of domestic animals, after flavour extraction.

So the new technology for paprika processing involves the more rational and more complete use of paprika for the production of spice concentrates and extracts.

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Obtainment of fish protein concentrate from low-price species

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ABSTRACT

In this paper a study is carried out about obtainment of fish protein concentrate from low-price species so as mackerel horse, *Trachurus trachurus* (L), mackerel, *Scomber scombrus* (L), and blue whiting, *Micromesistius poutassou* (Risso).

It is very important to become useful these species plentiful in Spain, obtaining from them commercial quality products and it is very interesting to know the behaviour of these species at different sets of usual manufactured methods of protein concentrate and stock problems.

The material obtained by us is high quality one and its characteristics place it higher in the type FAO-A.

The protein concentrate was used to enrich different human food with suitable results.

INTRODUCTION

One of the real aims of FAO at present is to obtain a good quality protein concentrate, stable with time and of a competitive price.

It is also known that fish can supply a protein concentrate of the best quality to compete with other similar products obtained from different raw materials.

So the logical need to use our fishery resources is covered in the most available way becoming them useful for the manufacturing of animal protein for human food.

OBJECTIVES

In this paper a study is carried out about obtainment of fish protein concentrate from low-price species so as mackerel horse, *Trachurus trachurus* (L), mackerel, *Scomber scombrus* (L), and blue whiting, *Micromesistius poutassou* (Risso).

We have used these species as raw material to get protein concentrate due to their low price, so the mackerel horse, *Trachurus trachurus* (L), of which is caught in Spain about 100.000 Tm per year, decreases sometimes till prices about 3 pesetas/kg. in sale-room.

Otherwise it is very interesting to know the behaviour of these species at different steps of usual manufactured methods of protein concentrate such as precoction, fat and humidity removal with organic

solvents and stock problems since the yield and manufacturing cost will undoubtedly depend on the original chemical characteristics of the raw fish.

RESULTS

Protein concentrate analysis from mackerel horse by different manufacturing methods.

We have used, to obtain protein concentrate fresh mackerel horse with the composition:

Humidity	75 %
Fat	5,67 %
Protein	19,40 %
Ashes	0,25 %

From this fish we essayed the manufacturing methods:

a) By extraction with isopropyl alcohol
b) By extraction with ethyl alcohol
c) Alkaline method, by prior treatment of fish with a solution of NaOH, 0,5N, at 60° C, a posterior precipitation of the protein with phosphoric acid and finally washing of the concentrate with ethyl alcohol.
d) Acid method, by previous treatment with 1 % SO H followed by another one with phosphoric acid and washing of the final product with ethyl alcohol.

Different protein concentrates obtained by these methods have been analyzed with the following results:

Mackerel horse protein concentrate

Method	Humidity %	Fat %	Protein %	Ashes %	Calories per 100 g humid weight
a.	4.4	0.00	90.5	3.14	371.1
b.	4.9	0.14	91.0	2.50	378.5
c.	3.3	0.59	96.0	0.71	399.1
cm.	3.7	0.00	94.4	2.10	387.0
d.	4.6	0.01	93.3	2.40	382.6

Likewise we can appreciate in every case the very high nutritive value of protein concentrate and its characteristics —low humidity and fat content— which let a storage without alteration in unlimited time.

Efficacy in the extraction with isopropyl alcohol at room temperatures, 40° C, 50° C, and 60° C.

In the Picture 1 we specify the results obtained —fat content, proteins and ashes— is the mackerel horse cooked and removed with isopropyl alcohol at different temperatures.

We see clearly that the efficacy in the removal is function of temperature so as when we work with dry fish the extraction is stronger.

Calculation of the yield of protein concentrate from fresh mackerel horse

For calculating this yield we have considered the protein concentrate method by extraction with isopropyl alcohol —cheaper solvent— which gives us a quality final product.

Yield	Median values % lost
Beheaded and eviscerated.	36
Coction.	14
Skinning and separation of bones.	17
Removal with solvent.	26

	Median values %
Final yield in protein concentrate from fresh mackerel horse with head and viscera.	7.0
Final yield in protein concentrate from beheaded and eviscerated fish.	11.2
Final yield in protein concentrate from clean and cooked fish.	21.5

These yields obtained in laboratory scale, can be better in semi-industrial or industrial scale using a suitable filter press equipment to avoid to loss the dry material during the extraction. On the other hand the manufacturing cost will decrease when we use rectification columns to recuperate the solvent.

PICTURE 1

Efficacy in the extraction with isopropyl alcohol at room temperature: 40° C, 50° C and 60° C

		Fat %	Proteins %	Ashes %
Mackerel horse cooked and removed at room temperature.	Raw	23.7	77.1	7.0
	1st extraction	19.9	81.4	6.6
	2nd extraction	2.2	97.5	7.4
	3rd extraction	0.3	97.9	8.7
Mackerel horse cooked and dried, removed at room temperature.	Raw	23.9	76.1	4.39
	1st extraction	5.0	92.2	4.68
	2nd extraction	0.9	98.1	4.58
	3rd extraction	–	98.7	4.38
Mackerel horse cooked and removed at 40° C.	Raw	19.5	79.8	5.1
	1st extraction	17.3	83.2	4.3
	2nd extraction	1.3	96.2	7.4
	3rd extraction	0.2	97.2	6.4
Mackerel horse cooked and removed at 50° C.	Raw	20.7	80.1	6.6
	1st extraction	12.9	87.9	4.9
	2nd extraction	1.1	98.1	5.5
	3rd extraction.	0.1	98.3	6.4
Mackerel horse cooked and removed at 60° C.	Raw	17.4	82.4	3.6
	1st extraction	8.4	93.1	3.8
	2nd extraction	0.9	98.2	3.7
	3rd extraction	–	98.8	4.0

(The values are referred themselves to dry weight.)

Determination of aminaocids in protein concentrate obtained from mackerel horse by isopropyl alcohol method

From protein concentrate the aminoacids were determinated by thin-layer chromatography and densitometry. The results obtained are:

Aminoacids	g/100 g of protein
Lysine.	12.8
Histidine.	2.4
Arginine.	6.0
Aspartic acid.	7.4
Glutamic acid.	13.6
Proline.	2.0
Glycine.	8.5
Alanine.	10.7
Valine.	3.2
Methionine.	3.2
Isoleucine + Leucine.	12.4
Tyrosine.	3.0
Phenylalanial.	3.3

Determination of trimethylamine. Peroxide index and bacteriological exam

The values obtained for us in the determination of TMA in protein concentrate from mackerel horse are about 2,1 mg. N-TMA/100 g. of product, what corresponds with a well keeped flour.

Likewise the values of peroxide index are null, what tells us there is not oxidation of fat substance.

We have made also a microbiological exam by plte count in progressive dilutions of total number of germens, using as nutritive means "*Bacto plate count agar of Difco*", with negative results, what show us that protein concentrate is practically sterile and it is free of pathogenic germens so as fungus which could spoil it later.

Use of other species of fish as raw material to obtain protein concentrate

We have essayed the isopropyl alcohol method to obtain protein concentrate from other fishes of different fat contents. The final products were similar to the ones obtained from mackerel horse and the analyses of immidiate principles gave us:

Analyses of protein concentrate obtained by isopropyl alcohol method from hake, blue whiting, mackerel and albacora: Yields

	Humidity %	Fat %	Proteins %	Ashes %	Yield about fish without skin nor bones(%)
Hake.	3.4	0.00	92.6	3.0	15.9
Blue whiting.	4.1	0.00	87.4	3.4	17.5
Mackerel.	3.1	0.12	94.7	3.3	18.8
Albacora.	3.7	0.08	90.1	3.7	24.1

Essays about enrichment of different foods with protein concentrate obtained from mackerel horse

We have enriched with protein concentrate from mackerel horse, different foods, so as bread, biscuits and chocolate. For this proof two factories have colaborated with us, one of them about bread and another about chocolate.

Tŏ manufacture bread and biscuits we have used 3 kinds of flours previously enriched with mackerel horse protein concentrate at 5, 10 and 20 %. In the chocolate using proportions of protein concentrate of 10, 20 and 30 % about the final product.

The organoleptical characteristics in these enriched foods were wright, without remarking in any case fish odour or taste. However the texture of bread changes giving a heavy product when the flour is enriched with 20 % of protein concentrate. This inconvenient can be solved using special aditives so as the glycerol monoestearate.

	Humidity %	Fat %	Proteins %	Ashes %	Calories
Bread without enriching.	27.6	0	9.2	1.95	37.72
Bread enriched with 5 % of protein concentrate.	28.4	0	11.8	2.00	48.38
Bread enriched with 10 % of protein concentrate.	32.6	0	13.7	1.94	56.17
Bread enriched with 20 % of protein concentrate.	33.5	0	19.0	1.92	77.90
Biscuits without enriching.	6.9	18.90	10.9	0.60	220.46
Biscuits enriched with 5 % of protein concentrate.	5.2	19.70	12.3	0.65	233.64
Biscuits enriched with 10 % of protein concentrate.	5.1	20.10	15.2	0.70	249.25
Biscuits enriched with 20 % of protein concentrate.	5.8	18.80	17.2	0.72	245.36
Chocolate without enriching.	0.85	29.80	4.7	1.18	296.41
Chocolate enriched with 10 % of protein concentrate.	1.25	32.00	9.7	1.16	337.37
Chocolate enriched with 20 % of protein concentrate.	1.80	32.00	16.1	1.13	363.61
Chocolate enriched with 30 % of protein concentrate.	2.20	32.70	20.9	1.02	389.80

In the organoleptical conditions of different enriched foods, the best results correspond to bread enriched with 10 % of protein concentrate, and chocolate and biscuits enriched with 20 % of protein concentrate.

We have made determinations of humidity, fat, proteins and ashes in different foods before and after enriching them with different proportions of mackerel horse protein concentrate.

We shaw the results obtained in the last picture.

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Production of an enzymic hydrolysate of casein

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ABSTRACT

An enzymic hydrolysate of casein free of bitter-tasting peptides has been developed by the use of papain and the cortical tissue of pig kidney. The original laboratory method has been slightly modified for the production of kilogram quantities. The bacterial count of the hydrolysate has been shown to be satisfactory and the biochemical composition has been investigated. The hydrolysate contains more than half of the amino acids in the free form and the remainder as small peptides. The nutritional value and potential use of this pre-digested protein for patients suffering from digestive disorders due to pancreatic insufficiency is discussed.

INTRODUCTION

Enzymic hydrolysates of protein have not been widely used as a source of pre-digested protein for patients suffering from pancreatic insufficiency which results in reduced digestive function for proteins and lipids. A limiting factor may have been unpalatability because of the occurrence of bitter tasting peptides in many partially hydrolysed proteins. The development of bitterness in some cheese which has undergone partial degradation of the protein is an example of this phenomenon, (Hamilton *et al.*, 1974). However, if the problem of bitterness could be overcome, enzymic hydrolysis has the advantage of incurring no alteration to the original amino acid composition of the substrate protein. On the other hand, hydrolysis of protein by enzymic treatment is unlikely to go to completion and yield a product composed totally of single amino acids; some peptide residues would survive the hydrolytic process. Recent work (Hellier *et al.*, 1972; Matthews, 1971; Silk *et al.*, 1973) indicates that some small peptides are absorbed intact from the gut and that there are separate transport mechanisms in the intestinal mucosa for free amino acids and for peptides. Therefore, the presence of small peptides in an enzymic hydrolysate may not make the product nutritionally inferior to a mixture of synthetic amino acids, or an acid hydrolysed protein, as a form of pretigested protein for patients with digestive malfunction. In fact, the possibility arises that an enzymic hydrolysate containing a mixture of free amino acids and small peptides

may have the additional advantage of resembling more closely the end products in the digestion and absorption of protein in healthy persons.

The objective of this project was to develop a protein product with a high degree of hydrolysis and free of the bitter taste. In the early stages of the investigation, varius casein products were hydrolysed with a range of proteolytic enzymes (Clegg and McMillan, 1974). Pig kidney tissue was found to contain enzyme systems capable of hydrolysing the bitter peptide which was released after the initial treatment of casein with an endopeptidase such as papain. A method for the production of an enzymic hydrolysate of casein has been based on this finding, initially on a laboratory scale and subsequently modified to produce kilogram quantities (Clegg *et al.*, 1974 a).

EXPERIMENTAL

Production in the laboratory

A 6.25 % aqueous suspension of commercial acid casein was adjusted to pH 6.4, 1 % papain was added and the mixture was incubated at 40° C for 18 hr. The pH was then adjusted to 8.0 and an aqueous homogenate of the cortical tissue of pig kidney, equivalent to 4 % of the substrate protein on a nitrogen basis, was added with a trace amount of Mn++ as enzymic activator. Incubation proceeded for a further 24 hr and then the hydrolysaste was boiled to remove the toluene which had been added as a preservative, to reduce the bacterial load, to coagulate any unhydrolysed protein, and to terminate enzyme activity. On cooling, the hydrolysate was centrifuged and the supernatant provided the final product which was freeze-dried.

Production with pilot plant equipment

Small modifications to the above procedure were required when the above method was scaled up to produce larger quantities of the hydrolysates. After the incubation with papain and kidney in a fermentation tank, a sieving operation was introduced to remove any intact protein or particles of kidney tissue. The hydrolysate was then pasteurised to terminate enzyme activity and to reduce the bacterial load before concentation of the solution to approximately 22 % solids in a falling film evaporator; chloroform was used as the preservative at the incubation stage and was removed during concentration. The concentrate was spray-dried and the powder had a maximum moisture content of 4 %. The results discussed below refer to batches of enzymic hydrolysate produced on this larger scale when starting with 12 kg granular casein.

RESULTS AND DISCUSSION

Microbiological evaluation

Bacteriological standards for this type of product are not laid down in Britain so, as a guideline, comparisons have been made with the West German regulations for infant foods. At an early stage, it was discovered that the papain was heavily contaminated and a change was made to an alternative commercial brand; papain from this second supplier, in addition to being bacterially cleaner, was more active and was used at a level of 0.5 % for the initial hydrolysis. The microbiological findings (Table 1) refer to batches of hydrolysate produced with this good quality papain. It will be seen that the microbiological quality of the hydrolysate is well within the standards for infant foods in West Germany except for the occasional presence of a very small number of anaerobic sporeformers. The kidney tissue does not present a microbiological hazard, especially considering its low level of addition relative to casein; in fact, the casein substrate was found to provide the greatest potential source of contamination for aerobic and anaerobic sporeformers. The possibility of the ingredients, or the final product, acting as carriers for *E. coli* is unlikely.

TABLE 1

Range of bacterial counts obtained during the production of 4 batches of enzymic hydrolysate of casein

Sample	Total count /1.0 g	Aerobic sporeformers /0.1 g	Anaerobic sporeformers /1.0 g	Coliform /0.01 g
Papain	< 10	< 10	0-5	Not detect.
Casein	3,500-19,000	11-710	0-80	Not detect.
Kidney	9,200-69,000	125-220	0-8	Not detect.
Hydrolysate	100-9,000	10-77	0-8	Not detect.
West German standards (max.)	50,000	150	Nil	Not detect.

Biochemical evaluation

After incubation of whole casein with papain (or with many other endopeptidases), the digest had a very bitter taste. This problem has been investigated and a bitter-tasting peptide was isolated from a papain digest of casein and identified as residue 53-79 inclusive of β-casein (Clegg *et al.*, 1974 b). The second stage of hydrolysis with the full spectrum of proteolytic enzymes occurring in kidney tissue had a de-bittering effect, as well as increasing the degree of hydrolysis, and the original bitter peptide was no longer present. The precise enzyme system in kidney tissue responsible for the hydrolysis of this proline-rich peptide has not been established but is thought to depend on the combined activity of leucine aminopeptidase, aminopeptidase-P and proline iminopeptidase (Lim, 1973).

Dialysis was used as a convenient and simple method to asses the extent of hydrolysis of the substrate casein into free amino acids and small peptides. A standardised technique with a solution of the hydrolysate was employed and the nitrogen diffusing through the tubing was expressed as a percentage of the total nitrogen originally contained in the dialysis sac. The percentage of dialysed nitrogen was taken as a measure of the free amino acids and provided an arbitary yardstick for the comparison of hydrolysates prepared with different enzymes or when experimental procedures were modified. On this basis, the digestion of casein with papain and followed by kidney tissue yielded hydrolysates which appeared to contain 46-53 % free amino acids. Further work has since shown this value to be an under-estimate.

Using our dialysis technique of suspending the open-ended tubing in a known volume of distilled water for exactly 24 hr, it was found that commercial mixtures of synthetic amino acids (Aminogran), a mixture of 8 amino acids prepared in the laboratory, and acid hydrolysed protein (Albumaid Complete) all yielded 71.6-72.9 % dialysable nitrogen, not 100 %. Assuming that a constant proportion of free amino acids are non-dialysable under these conditions, a factor (100/72 = 1.39) can be applied to the experimentally determined dialysable nitrogen values to give a more realistic assessment of the free amino acid content of a hydrolysate.

With a Sephadex G-10 column (86 x 2.2 cm) and eluting with a 1M solution of acetic acid, no amino acids were found in the first 150 ml eluant. Therefore, any nitrogen determined in fractions before this point must originate from larger molecules and be of peptide origin. Except for tyrosine and tryptophan which were eluted at approximately the 300 ml mark, the amino acids have no absorption at

280 nm. However, since a small amount of absorption was recorded in the 150-200 ml elution range for hydrolysates of casein, this fraction must contain some small peptides in addition to free amino acids. Therefore, to determine the free amino acids as the percentage of sample nitrogen which is recovered in the post-150 ml eluant yields an over-estimate.

Table 2 shows examples for the assessment of free amino acids obtained by gel chromatography, by direct analysis, and after correction of the dialysis figure. The corrected dialysis values of the hydrolysate are all less than the figures based on column chromatography which is in keeping with the observed failure to obtain complete separation between very small peptides and amino acids in the 150-200 ml fraction. The casein hydrolysate prepared by enzymic treatment with papain and kidney tissue probaly contains approximately two thirds free amino acids and one third small peptides, instead of the half and half division previously reported.

TABLE 2

The percentage of free amino acids in various protein products determined by column chromatography and dialysis

Sample	G-10 Sephadex column[a]	Dialysis	
		Direct	Corrected (x 1.39)
Aminogran.	97.8	72.9	101.3
Albumaid Complete.	97.2	72.1	100.2
Amino acid mixture.	97.2	71.6	99.5
Enzymic hydrolysate (1).	77.6	53.3	74.1
Enzymic hydrolysate (2).	76.2	45.9	63.8
Papain only, digest.	59.4	29.8	55.3

[a] $\frac{\text{N content of post-150 ml eluant}}{\text{N content of sample applied}} \times 100$

The overall amino acid composition of the enzymic hydrolysaste does not differ from that of casein because the dilution with a small quantity of kidney protein (4 % maximum) is not sufficient to produce any significant alterations in the levels of individual amino acids.

Nutritional evaluation

Small scale animal experiments showed, as expected, that the nutritional value of the enzymic hydrolysate of casein was the same (or slightly better) as intact casein for healthy growing rats (Clegg *et al.*, 1974 a).

Nitrogen balance studies with humans with defective pancreatic function due to cystic fibrosis or pancreatitis are in progress. Preliminary results show that pre-digested protein supplied by the hydrolysate has improved absorption and utilisation compared with the intact protein in a general diet under these physiological conditions.

The original problem of bitterness in enzymic hydrolysates has been largely overcome by including treatment with enzyme systems capable of splitting the bitter peptides responsible. Unfortunately, the final product is not completely bland to taste as it has an inherent flavour, possibly arising from the free amino acid content since these compounds are not without taste. The basic flavour of the enzymic

hydrolysate does not blend well with other flavouring agents, therefore it is often necessary for the patients to acquire a taste for the hydrolysate by perseverence. The spray-dried powder is reconstituted with water to a puree or thinner drink according to taste.

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Protein isolate from colombian cottonseed meals

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ABSTRACT

Protein isolates of acceptable organoleptic quality were obtained using Colombian cottonseed meal. The protein was extracted using two different solutions: sodium hexametaphosphate (I) and sodium hydroxide (II), followed by acid precipitation. The first isolate (I) contained 90 % protein and because of it creamy color could be used as a protein supplement at the 5 % level in bread, corn, cakes, gruels, and soups. The second isolate (II), with equal protein content was dark tan, which unfortunately limited its uses. Yields were better using sodium hydroxide as on extracting solution. Approximate cost per kilo is similar to that of soy protein isolate in Colombia.

INTRODUCCIÓN

El método de extracción del aceite por prensado-solvente, de uso común en Colombia, permite producir tortas bajas en gosipol libre y con nivel aceptable de lisina disponible si se regulan adecuadamente las condiciones del proceso (Buckle et al. 1971). Sin embargo, aunque el gosipol se inactive durante el proceso, permanece en el producto junto con otros pigmentos e imparte un intenso color amarillo-verdoso a las mezclas de alimentos en que la torta es incorporada como suplemento proteínico (Milner 1964). Esto constituye un gran problema de aceptabilidad que limita el aprovechamiento de este tipo de tortas para consumo humano. Por consiguiente, las tortas de algodón solo se utilizan en Colombia en la industria de alimentos para animales, a pesar de que en los últimos años se han presentado excedentes de producción, los cuales han sido estimados para 1974 en 20,800 toneladas (Ministerio de Agricultura, Colombia, 1973). Entre los productos derivados de la semilla de algodón disponibles en la actualidad y procedentes de distintos procesos comerciales, o en experimentación, el de mejor calidad corresponde a la harina procedente de semilla de algodón sin gosipol, único producto que no presenta problemas de color (Vix et al. 1969). Esta variedad no existe en Colombia y no se presume introducción en un futuro cercano.

En los trabajos efectuados en los últimos años para la producción de concentrados o aislados de proteína de algodón, la tendencia ha sido la de utilizar como materia prima semillas o harinas de algodón con o sin gosipol, obtenidas por procesos en los cuales las tortas no sufren tratamientos térmicos fuertes, como los

que reciben las tortas en el proceso prensado-solvente, utilizado por la industria en Colombia. El proceso de ciclones líquidos desarrollado por el U. S. D. A. (Ziemba, 1973), basado en la separación física de las glándulas de gosipol y que ya está siendo aplicado a escala industrial, utiliza como materia prima la semilla de algodón y produce un concentrado con 66 % de proteína. En Colombia no hay excedentes de semilla y aparentemente la introducción del proceso de ciclones líquidos no presenta posibilidades favorables por el momento.

Estos hechos mostraron la necesidad de desarrollar un proceso que permitiera usar en alimentación humana los crecientes excedentes de torta de algodón colombiana y llenar en parte el déficit de proteínas disponibles para alimentación humana en el país.

En este trabajo se adaptaron para las tortas comerciales prensado-solvente, dos de los procesos disponibles desarrollados en los últimos años para introducir aislados de proteína, que utilizan como agentes extractores hidróxido de sodio (Berardi et al. 1969) y hexametafosfato de sodio (Shemer et al. 1973).

PARTE EXPERIMENTAL

Tortas de Algodón

Se utilizaron tortas de algodón comerciales obtenidas por el proceso de prensado-solvente. Las tortas fueron molidas en un molino Raymond N.° 82 utilizando una malla de 0.024 pulgadas de abertura. En la Tabla 1 se muestra en análisis químico de las tortas empleadas en los ensayos. Como agentes extractores se usaron hexametafosfato de sodio, calidad técnica e hidróxido de sodio, reactivo analítico.

TABLA 1

Composición de las tortas de algodón

Determinación	Tortas		
	A	B	C
Humedad, %.	10'1	10'54	9'84
Grasa, %.	0'43	1'30	0'86
Fibra cruda, %.	8'22	11'49	13'02
Cenizas, %.	7'37	7'12	6'22
Nitrógeno, %.	7'92	6'86	7'19
Nitrógeno soluble en NaOH 0'02 N, %.	59'4	75'6	68'2
Gosipol libre, %.	0'05	0'05	0'02
Gosipol total, %.	1'00	0'87	0'70
Lisina disponible, g/16 g de N.	3'88	4'0	3'76
Triptofano, g/16 g de N.	1'36	–	–
Metionina, g/16 g de N.	1'40	–	–

Procesos:

Los métodos seleccionados para aislar la proteína fueron el de extracción con hexametafosfato de sodio (Proceso 1) y extracción con hidróxido de sodio en una sola etapa (Proceso 2) con precipitación de la proteína en medio ácido. En ambos casos se estudiaron las variables que afectan el rendimiento y la calidad de los productos, tales como concentración de la solución extractora, pH de la solución extractora, tiempo y temperatura de extracción, pH de precipitación, etapas de lavado y ajuste del pH del producto final. La relación harina solvente fue en todos los casos de 1 : 10.

Rendimiento de Extracción.

Se tomaron 100 g de harina y se suspendieron en un litro de la solución extractora, en un vaso de precipitados colocado dentro de un baño de agua a la temperatura adecuada. La suspensión se mantuvo en agitación durante el tiempo del ensayo. Se centrifugó y se analizó la cantidad de nitrógeno en el sobrenadante. El rendimiento de extracción se calculó de la siguiente manera:

Rendimiento de extracción % =

$$\frac{\text{g de nitrógeno en solución}}{\text{g de nitrógeno en la torta original}} \times 100$$

Rendimiento de Precipitación.

Extractos de proteína en soda y en hexametafosfato de sodio fueron acidificados con ácido clorhídrico a varios valores de pH. Se separaron los precipitados por centrifugación y se determinó la cantidad de nitrógeno que quedó en el sobrenadante. El rendimiento de precipitación se calculó de la siguiente manera:

Rendimiento de precipitación % =

$$\frac{\text{g de N en el extracto} - \text{g de N en el sobrenadante}}{\text{g de N en el extracto}} \times 100$$

Para estudiar la influencia de la temperatura y el pH de extracción sobre la calidad del aislado se hicieron extracciones con hexametafosfato de sodio al 2 % a 40 y 60° C y pH de 6.2 y 9. La proteína se precipitó a pH 2.3, se liofilizó y se hicieron determinaciones de proteína total y lisina disponible en los aislados.

Finalmente se llegó a la adopción de los siguientes procesos para aislar la proteína en planta piloto.

Proceso 1 - Extracción de la proteína con solución de hexametafosfato de sodio al 2 %, separación del extracto por centrifugación, precipitación de la proteína con ácido clorhídrico hasta pH 2.3, ajuste del pH del precipitado hasta pH 5.5, lavado con agua y secado por atomización (ver figura 1).

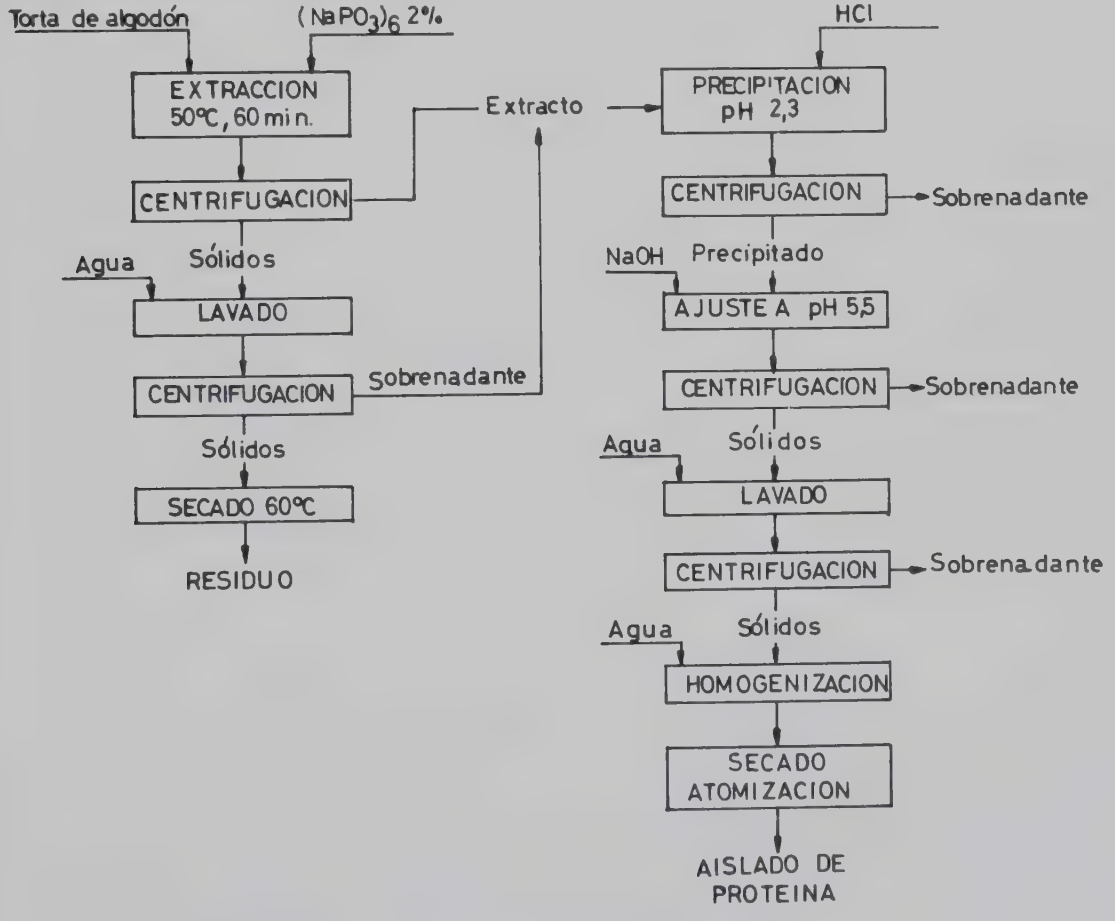


Fig. 1.–Diagrama de flujo. Proceso I.

Proceso 2 - Extracción de la proteína con NaOH 0.04 N, separación del extracto por centrifugación, precipitación de la proteína con ácido clorhídrico hasta pH 5.0, lavado con agua y secado por liofilización en el laboratorio y por atomización en planta piloto (ver figura 2). En el laboratorio se trabajó con 200 gramos de harina de algodón y en la planta piloto con 10 y 20 kilos. Se utilizaron los siguientes equipos: marmita Lee con agitación, centrífuga De Laval Modelo BRPX207-20SE, molino coloidal Fryma, secador de bandejas con circulación de aire caliente y atomizador Niro N.° 1200.

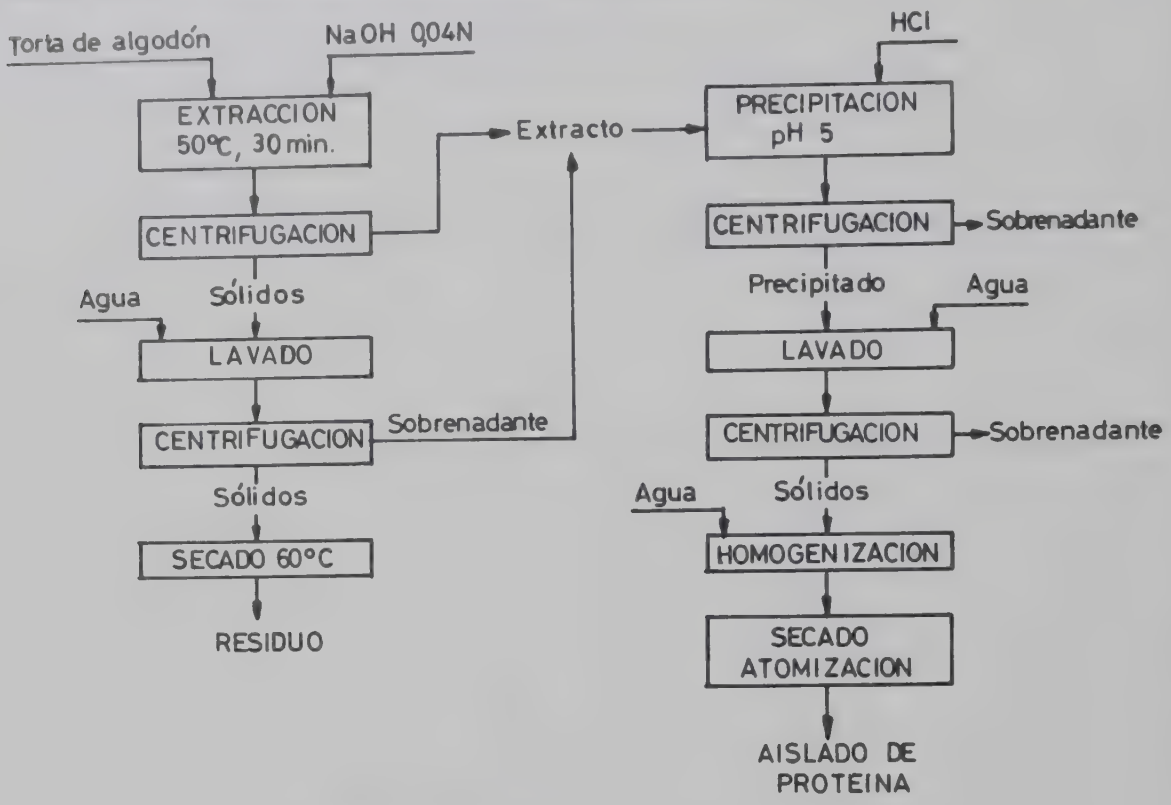


Fig. 2.–Diagrama de flujo. Proceso II.

Métodos Analíticos.

Las determinaciones de humedad, grasa, fibra cruda, cenizas se efectuaron según métodos estandar (AOAC 1970). El nitrógeno se determinó por el método Kjeldahl, la lisina disponible según el método de Conkerton et al. (1959), proteína soluble en soda 0.02 N según Lyman et al. (1953), gosipol libre y total por los métodos AOCS Ba-7-58 y Ba-8-55. La metionina se analizó despues de hidrólisis ácida (24 horas HCl 6 N) en una analizador automático de aminoácidos EEL del TPI de Londres. Para el triptofano se utilizó el método de Miller (J. Sc. Fd. Agric. 18 : 381 1967). El color de los aislados fue medido en un colorímetro diferencial Hunter Modelo D25. Para determinar la solubilidad de la proteína de los aislados se prepararon buffers de citrato-fosfato y se ajustó en cada caso la fuerza iónica a un valor constante de 0.6, agregando KCl. Se dispersaron 4 g de muestra en 80 ml de la solución buffer, la suspensión se agitó durante 20 minutos y luego se centrifugó durante 10 minutos. Se determinó la cantidad de nitrógeno que queda en solución.

RESULTADOS Y DISCUSIÓN

Extracción

El efecto de la concentración de hexametafosfato de sodio y el pH de la solución extractora se aprecia en la figura 3. No se observa un aumento en los rendimientos al elevar la concentración de hexametafosfato de sodio por encima del 2 % ni al variar el pH de la solución extractora entre 6 y 11.

La temperatura aumenta los rendimientos de extracción. Un aumento de 40 a 60° C produce un incremento del 5 % en el rendimiento. El tiempo de extracción tuvo un efecto menor. Shemer et al. (1973) reportaron resultados similares

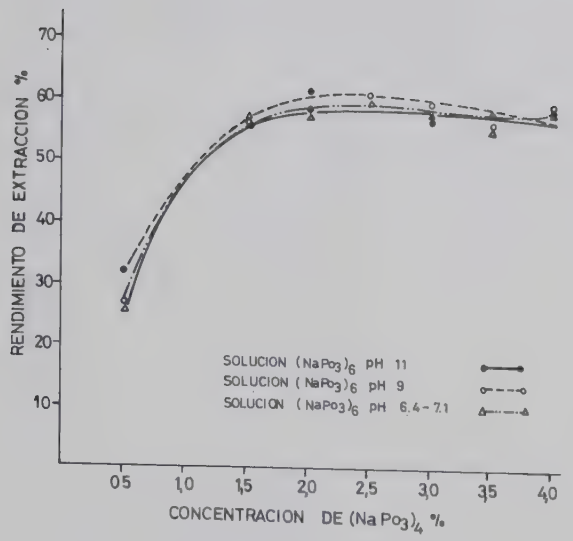


Fig. 3.–Efecto de la concentración de hexametafosfato de sodio y de pH sobre el rendimiento de extracción.

trabajando con harinas de algodón obtenidas por el proceso de extracción con solvente en dos pasos.

En el caso de la extracción con hidróxido de sodio, el principal factor que influyó en el rendimiento de extracción fue la concentración de soda, como se observa en la Tabla 2. Berardi et al. (1969) encontraron que para una máxima extracción del nitrógeno con hidróxido de sodio, se requieren 0.4 meq de base por gramo de harina. En efecto, utilizando soda 0.04 N (relación harina-solvente 1:10) se logró extraer casi el 70 % de la proteina inicial mientras que con una concentración 0.02 N no se alcanzó a llegar al 50 %.

La temperatura de extracción influyó principalmente cuando se empleó soda 0.02 N, ya que un aumento de 30 a 50° C produjo un incremento del 12 % en el rendimiento. El aumentar el tiempo de extracción por encima de 20 minutos no produjo una mejora apreciable en el rendimiento de extracción.

TABLA 2

Efecto de las condiciones de extracción sobre rendimiento - Proceso 2

Concentración de NaOH	Tiempo (min.)	Temperatura (°C)	Rendimiento de extracción (%)
0'02 N	20	30	35'3
0'02 N	40	30	35'3
0'02 N	20	50	47'1
0'02 N	40	50	48'6
0'04 N	20	30	65'9
0'04 N	40	30	67'3
0'04 N	20	50	69'3
0'04 N	40	50	69'8

Precipitación

En la figura 4 se aprecian las curvas de precipitación de proteína para los extractos de hexametafosfato de sodio y en hidróxido de sodio. En el primer caso, el mínimo de proteína en solución (rendimiento de precipitación 88-89 %) ocurre a un pH entre 2 y 3. A pH 2.3 se observó una completa floculación del precipitado, por

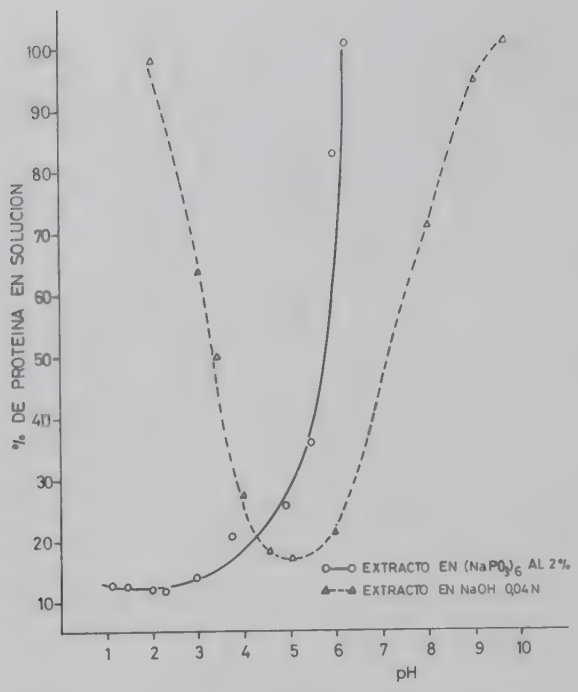


Fig. 4.–Curvas de precipitación de proteína.

lo cual éste fue el pH de precipitación seleccionado para el proceso 1. Para el proceso 2, el máximo rendimiento de precipitación se obtuvo aproximadamente a pH 5, lo cual coincide con los resultados obtenidos por Berardi et al. (1969) para la extracción con soda en un sólo paso.

Los distinos valores de pH de mínima solubilidad para los procesos 1 y 2 puede explicarse por la diferente fuerza iónica de las soluciones extractoras, ya que el punto isoeléctrico de las proteínas varía con la fuerza iónica del medio y se desplaza hacia el lado ácido cuanto mayor sea ésta (Fox et al. 1957). La solución de hexametafosfato de sodio al 2 % (0.036 M) tiene una fuerza iónica varias veces superior a la solución de soda 0.04 N.

Rendimiento de los Procesos:

En la Tabla 3, se puede observar que los factores principales que afectan el rendimiento total en los procesos son la no solubilización de proteína en la etapa de extracción (residuo insoluble), la pérdida debida al extracto que queda impregnado en el residuo no soluble (nitrógeno no separado del residuo) y las pérdidas generales de operación.

En el proceso 2, se obtuvieron rendimientos de extracción mas altos (64-67 %) que en el proceso 1 (53-57 %), lo que indica una mayor eficiencia de la solución de hidróxido de sodio para extraer la proteína. Berardi et al. (1959) obtuvieron rendimientos de extracción de 82.8 % en el proceso de extracción con soda en un solo paso, para harinas de algodón sin gosipol. Igualmente, se han obtenido rendimientos hasta de 86 % usando hexametafosfato de sodio, para harinas obtenidas por extracción con solvente en dos pasos (Shemer et al. 1973). En ambos casos se trabajó con harinas de algodón prácticamente no desnaturalizadas. Los rendimientos de extracción de proteína inferiores obtenidos para las tortas de algodón prensado-solvente, se deben al fuerte tratamiento térmico sufrido durante el proceso de extracción del aceite en condiciones industriales, que produce una disminución de la solubilidad de la proteína (Altschul, 1958) (Buckle, et al. 1971).

La introducción al proceso de una etapa de lavado del residuo insoluble, disminuyó notablemente la cantidad de proteína soluble que queda impregnando en el residuo (Ensayo I y VI).

TABLA 3

Rendimientos y balance de proteína

Proceso	Ensayo	Rendimiento		% del nitrógeno total							
						Sobrenadante					
		Extracción %	Precipitación %	Residuo insoluble	No separado del residuo	Precipitación	Lavado precipitado	Ajuste pH	Lavado precipitado	Pérdidas de operación	Aislado de proteína
1	I.–Laboratorio 200 g torta B.	52'8	85'9	47'1	4'5*	7'8		7'2	1'6	1'9	34'4
	II.–Planta piloto 10 kg torta A.	53'3	87'0	46'7	12'9	4'2	0'4	1'0	1'6	16'6	16'6
	III.–Planta piloto 10 kg torta A.	56'7	89'3	43'3	15'6	4'1	0'1	1'8	1'2	17'1	16'8
	IV.–Planta piloto 20 kg torta B.	–	–	–	–	–	–	–	–	–	25'8
2	V.–Laboratorio 200 g torta C.	67'8	82'4	32'2	19'5	8'5	1'5			5'1	33'2
	VI.–Planta piloto 20 kg torta A.	64'4	84'2	35'6	4'7*	9'4	1'9			17'9	35'2

* Con lavado del residuo.

TABLA 4

Composición química de aislados y residuos

	Humedad %	Grasa %	Fibra cruda %	Ceniza %	Nitrógeno %	Fósforo %	Gosipol		Lisina Disponible g/16 g N	Metionina	Triptófano
							Libre	Total			
Aislado proceso 1*.	8'1	0'2	0'3	7'7	14'6	3'0	0'01	0'09	3'3	1'1	1'29
Aislado proceso 2*.	7'4	0'3	0'8	1'9	14'4	–	0'01	0'58	3'2	–	–
Residuo proceso 1**.	8'0	0'7	22'6	3'9	5'5	–	0'04	1'5	4'0	1'7	1'17
Residuo proceso 2*.	8'0	0'2	18'2	12'1	5'3	–	0'04	1'3	3'74	–	–

* Torta A. ** Torta B.

Se hicieron algunos ensayos (II y III) en el proceso 1, lavando el precipitado antes de ajustar el pH, para disminuir de esta manera la cantidad de proteína que se redisuelve en esta operación. Sin embargo, el aumento obtenido en el rendimiento total fue muy bajo como para justificar la introducción de una nueva etapa. Por esta razón este lavado se eliminó del proceso.

Como subproducto de la extracción se obtienen residuos equivalentes al 50 % del peso de la torta original, cuyas características se presentan en la Tabla 4 junto con las de los aislados para ambos procesos.

Características de los aislados y de los residuos:

El contenido de nitrógeno es similar para los aislados obtenidos por los procesos 1 y 2, equivalente a niveles de proteína de 90 % (N x 6.25). Las cenizas para el aislado del proceso 1 son bastante superiores a las del aislado del proceso 2, posiblemente debido a la presencia de fosfatos residuales. El gosipol libre para ambos aislados es del orden de 0.01 %, nivel muy inferior al máximo recomendado por WHO/FAO/UNICEF (Milner 1962), para harinas de algodón utilizadas para consumo humano. El contenido de gosipol total del aislado del proceso 2 es de seis veces superior al del aislado del proceso 1. La lisina disponible en los aislados, disminuyó con relación a la de la torta original. Los análisis practicados sobre el aislado del proceso 1, indicaron que también bajan las concentraciones de metionina y triptofano. Se encontró que para el caso de la lisina disponible esta disminución no está relacionada con la temperatura y el pH en la etapa de extracción, según se puede ver en la Tabla 5, ya que con temperaturas de 40 y 60° C y pH de 6.2 y 9.0, se obtuvieron niveles similares de lisina disponible en los aislados. Shemer et al. (1969) recomendaron no elevar la temperatura de extracción por encima de 55° C, con el fin de evitar el daño en el valor nutritivo de la proteína por disminución del contenido de lisina disponible. Sin embargo, parece que en el caso de las tortas prensado-solvente, el factor dominante en esta disminución es una distribución no uniforme de los aminoácidos en las distintas fracciones resultantes del proceso, similar a la observada para aislados de proteína de soya precipitados con ácido (Smith et al. 1972).

TABLA 5

Influencia de las condiciones de extracción sobre el contenido de lisina disponible - Proceso 1

Temperatura	pH	Aislado Lisina disponible g/16 g de N
40	6'2	3'29
40	9'0	3'28
60	6'2	3'26
60	9'0	3'20
Torta original		3'68

Los residuos de extracción para los dos procesos presentan características químicas similares. Los contenidos de proteína son del orden de 34 % (N x 6.25) y los de fibra de 18-22 %. Las otras características son similares a las de la torta original, lo que haría de estos residuos ingredientes aprovechables en alimentación animal.

Propiedades funcionales:

Los aislados secados por atomización presentan sabor neutro, sin olor, y un color crema para el aislado del proceso 1 y marrón claro para el del proceso 2. Los aislados obtenidos en el laboratorio y secados por liofilización tienen colores más claros. En la Tabla 6 se presentan los resultados de las medidas de color Hunter. Los valores obtenidos para el aislado del proceso 1 son más cercanos al patrón de color blanco. La diferencia total de color con relación a este patrón es inferior para los aislados del proceso 1. Se observa que el tipo de secado influye sobre el color final de los aislados con mayor intensidad para el caso del aislado del proceso 2, especialmente en cuanto al parámetro L, indicativo del grado de blancura. El color más oscuro del aislado del proceso 2 coincide con un contenido de gosipol total muy superior.

TABLA 6

Color de los aislados

	L	a	b	ΔE*
Aislado proceso 1, liofilizado.	88'7	–1'2	15'5	17'7
Aislado proceso 2, liofilizado.	80'1	1'0	17'0	21'1
Aislado proceso 1, atomizado.	80'2	–1'4	21'7	24'9
Aislado proceso 2, atomizado.	55'7	5'7	19'6	42'5
Patrón de color blanco.	93'1	–0'6	0'4	

*$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$, calculado con relación al patrón de color blanco.

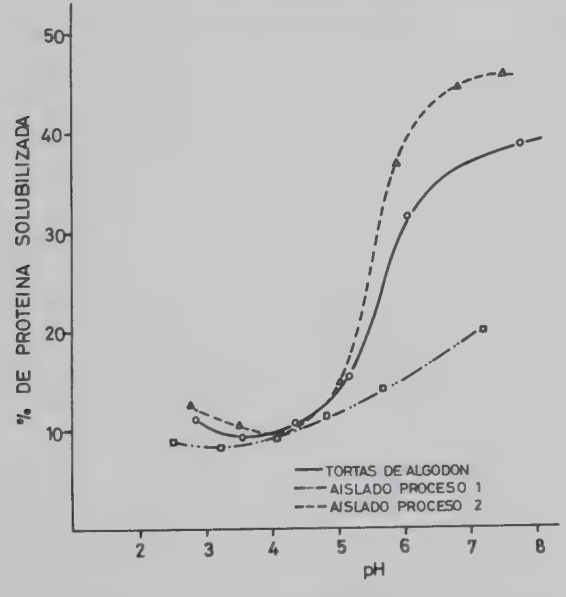


Fig. 5.–Curvas de solubilidad de proteina.

Las curvas de solubilidad de los aislados y de la torta original, entre pH 2 y 8 se encuentran en la figura 5. El aislado del proceso 2 presenta solubilidad más alta que el del proceso 1 y que la torta original. La diferencia en la solubilidad entre los dos aislados parece ser debida a los distintos pH de precipitación (Lawhon et al. 1971).

El aislado del proceso 1 fue ensayado como enriquecedor a niveles del 5 % en algunos productos de consumo popular en Colombia, como pan, arepas, coladas y sopas. Se encontraron resultados aceptabes en todos los casos, demostrándose que

no se deteriora el color de estos alimentos y que se ha obviado en esta forma el problema principal que presentan como enriquecedores otros productos de algodón. El aislado del proceso 2, por su tonalidad marrón presenta limitaciones en su aplicación en alimentos. Podría ser utilizado en productos de tonalidades oscuras, en mezclas con chocolate, en galletas y sopas, pero no en productos tales como pan y arepas.

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Using indigenous flours and starches as bread supplements in developing countries

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ABSTRACT

The study was conducted to explore whether or not bread would be acceptable if supplemented with high percentages of nonwheat flours or starches. Supplements could cause adverse effects on dough properties and bread quality. However, by using a modified breadmaking process and adding a surfactant, (ethoxylated monoglycerides, sodium stearoyl-2 lactylate or sucrose tallowate), we prepared acceptable bread from wheat flour supplemented with up to 20 % nonwheat flour or starch from several crops indigenous to developing countries. Soy flour, added at 5 or 8 %, could effectively raise the supplemented bread's protein content and protein quality, measured by amino acids' chemical scores. Supplemented bread firmed rapidly during storage. Surfactants helped stored breads retain softness.

INTRODUCTION

In many countries like Brazil and Indonesia, where the supply of nonwheat flours and starches from indigenous crops is abundant, use of locally available flours and starches is important. Bread is an increasingly popular, staple food among peoples in many developing countries. If indigenous flour or starch can replace part or all of wheat flour for making acceptable bread, the value of some native crops would be greatly increased. Considerable progress has been made in developing new methods with surfactants or gums to produce breads from wheat flour supplemented with nonwheat flours or starches by such investigators as Rotsch, 1953; Jongh, 1961; Kim and De Ruiter, 1968; Pringle et al., 1969; Dendy et al., 1970; Perten, H., 1970; and Hamed, et al., 1973. However, loaf volume and crumb grain of such breads require further improvements to make the bread acceptable.

We, therefore, studied improving effects of surfactants on the baking

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performance of wheat flour supplemented with nonwheat flour or starch and also the shelf life of supplemented breads, along with protein and amino acid contents of such breads.

EXPERIMENTAL

Materials

We used several nonwheat flours and starches, and two commercially available wheat flours (malted and bromated) (Table 1). Corn flour was milled from high lysine corn in the department. Sodium stearoyl-2 lactylate (SSL), calcium stearoyl-2 lactylate (CSL), and ethoxylated monoglycerides (EM), and sucrose tallowate used are products of C. J. Patterson Company and Colonial Sugar Company.

TABLE 1

Proximate analyses of wheat flours, and nonwheat flours and starches

Sample	Moisture %	Protein %	Ash %	Crude fat %	Carbohydrate (calculated) %
Flour:					
Cassava.	6.2	2.4	1.40	0.50	89.5
Corn.	15.3	6.0	0.24	0.30	78.2
Rice.	9.9	7.8	0.31	0.04	82.0
Rye.	10.3	18.9	4.10	0.50	66.2
Sorghum.	9.8	9.4	0.33	—	—
Soy.	6.4	52.6	7.20	0.50	33.3
Wheat I.	12.6	11.7	0.42	—	—
Wheat II.	13.7	12.7	0.48	—	—
Yam.	9.8	5.2	2.07	0.40	82.5
Starch:					
Cassava.	15.2	0.9	0.21	0.02	83.7
Corn.	11.7	0.7	0.06	0.09	87.5
Potato.	14.8	0.4	0.29	0.10	84.4

Analyses

Proximate analyses of flours and starches were as described in A. A. C. C. Methods, 1962, except that fat was determined by the A. O. C. S. Method (Aa 4-38), 1971, using petroleum ether as extracting solvent. Protein contents for all samples were converted by N x 6.25, except N x 5.7 for wheat flours (Table 1).

Amino acids were determined by the procedure of Waggle et al., 1966. Bread samples after (air) drying and grinding were hydrolyzed in 6N HCl for 22 hr. Essential amino acids were measured in a Beckman 120B amino acid analyzer. Methionine and cysteine were calculated as outlined by Moore, 1963, with a 24 hr. oxidation period, and hydrolyzed for 18 hr. No corrections were made for possible destruction of labile amino acid.

Farinograms

Farinograms were obtained by the constant-dough weight method, using 50 g. of sample (A. A. C. C. Methods, 1962).

Baking test

The K-State Process for making high-protein bread (Tsen and Tang, 1971) was used. 40 p. p. m. bromate was used for all the baking tests. Loaf weight and volume, averaged from duplicates, were measured withing 10 min. after bread was removed from the oven. Specific loaf volume (c.c. per g.) was

then calculated from the average loaf weight and volume. Specific loaf volume is important in marketing bread. In the U. S. A., specific volume of marketable bread (1- ¿b. loaf) appearance, crumb texture, and grain. Bread was scored from 1 to 10, 18 hr. after baking. That scored below 5 was regarded as unsatisfactory. Most of the baking tests were repeated at least once on a different day to substantiate results.

Unless otherwise indicated, amount of nonwheat flour or starch and other ingredients added to wheat flour for baking tests and farinographic studies were expressed as percentage of wheat flour (baker's percentage).

Storage test

Staleness (firmness) was tested by following changes in crumb firmness with a Bloom gelometer with a 1-in. plunger during storage. Three 1-in. slices were taken from each of two loaves which had been wrapped, sealed, and stored at room temperature (about 25° C) for 1, 3, or 5 days. Three (top, center, and bottom) gelometer (compressimeter) readings (g.) were taken on each slice.

RESULTS AND DISCUSSION

Farinographic properties of wheat flour supplemented with nonwheat flours and starches

Table 2 summarizes the farinographic properties of wheat flour supplemented with rice flour, corn flour, corn starch, cassava flour, or cassava starch. Rheological properties of dough changed drastically when some wheat flour was replaced by nonwheat flour or starch. Developing time and stability were sharply reduced, indicating that nonwheat flour or starch weakened dough structure by diluting wheat gluten. The reduced developing time and stability vary with different flours or

TABLE 2

Farinographic properties of wheat flour I supplemented with indicated percentages of corn flour, corn starch, cassava flour, cassava starch, or rice flour

Supplement %	Absorption %	Developing time min.	Stability min.
0.	63.0	7.00	10.70
Corn flour			
20.	62.9	4.80	9.50
30.	60.0	3.95	7.60
40.	59.3	3.15	6.85
Corn starch			
20.	61.3	2.40	7.00
30.	60.7	1.65	4.65
40.	60.4	1.25	2.90
Cassava flour			
20.	64.2	5.50	7.00
40.	65.0	4.50	4.50
Cassava starch			
20.	62.0	6.00	7.25
40.	60.4	5.50	7.00
Rice flour			
20.	63.3	4.45	6.90
40.	64.1	4.00	4.80

starches. In fact, Rasper et al. (1974) have found that yam, cocoyam, or platain starches can increase developing time significantly.

Improving effect of surfactants

Surfactants such as glyceryl monostearate and CSL effectively improve the baking performance of nonwheat flours or starches (Jongh, 1961; Kim and De Ruiter, 1968). It was known that surfactants like sucrose ester, SSL, or EM were required to produce acceptable high-protein bread from wheat flour fortified with soy flour or other protein-rich additives (Pomeranz, 1969; Tsen et al., 1971, and Tsen and Hoover, 1973). We, therefore, conducted experiments to see whether or not sucrose esters, SSL, or EM would improve dough properties of doughs prepared from wheat flour supplemented with 10 to 50 % corn starch (Table 3).

SSL added at 0.5 % substantially increased developing time and stability. EM, though less effective than SSL, also increase developing time. However, it raised the stability of doughs only when they were supplemented with more than 40 % corn starch. Different reactions from SSL and EM presumably result from SSL being an ionic surfactant while EM is a nonionic surfactant. Sucrose tallowate, also a monionic surfactant, and EM affect dough similarly. The dough strengthening effects of SSL, EM, and ST seem responsible, at least in part, for their improving the baking performance of wheat flour supplemented with nonwheat flour or starch.

TABLE 3

Effect of surfactants on the developing time and stability of doughs prepared from wheat flour II supplemented with corn starch

Corn starch % added	Surfactant 0.5 % added	Developing time, min.	Stability min.
0.	0	10.00	15.05
10.	0	7.00	13.75
30.	0	2.25	10.40
50.	0	1.25	4.20
0.	SSL	15.00	24.40
10.	SSL	10.50	17.85
30.	SSL	6.00	14.20
50.	SSL	5.40	11.30
0.	EM	10.00	14.05
10.	EM	8.00	12.90
30.	EM	4.70	9.60
50.	EM	3.00	5.05

Breads from wheat flour supplemented with nonwheat flour or starch

Effectiveness of surfactants, including EM, CSL, SSL, and ST, added at 0.5 % (on flour basis) were compared for improving baking performance of wheat flour supplemented with 20, 30, or 40 % corn flour or corn starch (Table 4 and Figures 1 and 2).

Acceptable breads with a specific loaf volume of more than 6.00 c.c./g. and a grain score higher than 5 were prepared from wheat flour supplemented with up to 20 % corn flour. Under our experimental conditions, corn starch could be suplemented up to 30 % (Tables 4 and 5 and Figs. 1 and 2). Corn starch was better supplement than corn flour.

TABLE 4

Effects of surfactants on volume (cc/g) and grain score (in parentheses) of breads made from wheat flour I supplemented with indicated percentages of corn flour or starch

Supplement	Surfactants, 0.5 % added				
	0	Em	CSL	SSL	ST
Corn flour					
20 %.	4.32(4)	6.23(8)	4.92(5)	6.01(8)	6.25(8)
30 %.	3.91(3)	5.57(7)	4.43(4)	5.25(6)	5.58(7)
40 %.	3.10(2)	4.94(6)	4.53(4)	4.89(5)	5.11(6)
Corn starch					
20 %.	4.54(4)	6.42(8)	5.29(8)	6.25(8)	6.55(8)
30 %.	4.49(4)	6.02(7)	5.20(7)	5.49(7)	6.14(7)
40 %.	4.30(3)	5.85(6)	4.39(6)	4.55(6)	5.67(6)

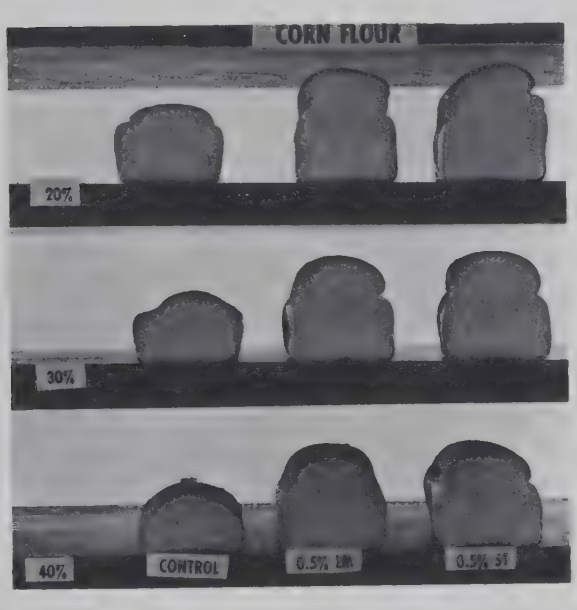


Fig. 1.–Breads from wheat flour fortified with 20, 30, or 40 % corn flour and treated with 0.5 % EM or ST.

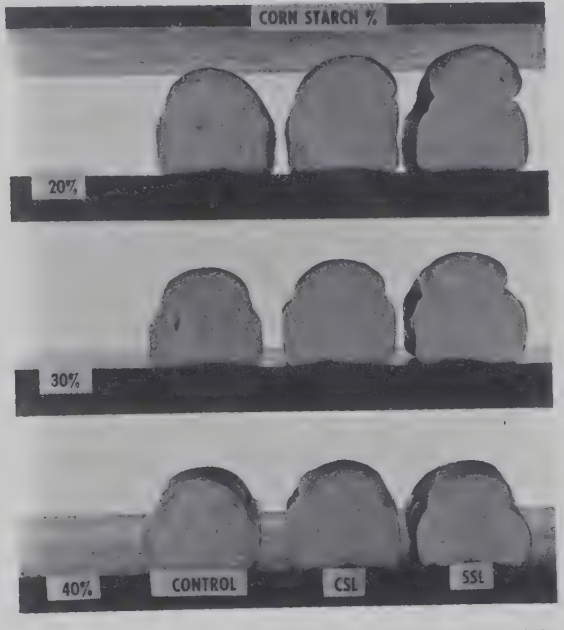


Fig. 2.–Breads from wheat flour fortified with 20, 30, or 40 % corn starch and treated with 0.5 % CSL or SSL.

In addition to corn flour and corn starch, we tested several other nonwheat flours and starches as bread supplements (Table 5). Among them, cassava starch and rice and yam flours gave large loaves; others also gave respectable loaf volumes. Loaf volume, of course, can be raised or lowered by using wheat flours of various baking qualities.

Specific loaf volume of 6.00 c.c./g. in generally used to gauge regular white bread's marketability in the U. S. The standard is arbitrary. Breads with specific volumes of more than 4.50 are acceptable in many countries. With 4.50 as the standard, up to 30 % nonwheat flour or starch could be used by adding 0.5 % EM, SSL, or ST.

TABLE 5

Specific loaf volume and grain score of breads prepared from wheat flour and wheat flour II fortified wirh 20 % nonwheat flour or starch

Supplement	No SSL		0.5 % SSL	
	Avg. sp. vol. cc/g	Grain score	Avg. sp. vol. cc/g	Grain score
Control.	6.46	8	7.18	9
Cassava flour.	5.40	5	5.87	8
Cassava starch.	5.37	5	6.01	9
Sorghum flour.	4.35	4	5.87	6
Rye flour.	4.01	5	5.10	7
Yam flour.	5.25	5	6.11	8
Rice flour.	4.49	5	6.40	9

TABLE 6

Volume and grain score of breads from indicated blends of wheat flour II, and nonwheat flours or starches treated with 0.5 % SSL or ST

Supplement %	Wheat flour %	Soy flour %	Surfactant 0.5 % added	Avg.sp.vol. cc/g	Grain score
Corn flour					
20.	75	5	SSL	6.01	8
20.	72	8	SSL	5.29	7
20.	75	5	ST	6.13	7
20.	72	8	ST	5.90	7
Rice flour					
20.	75	5	SSL	6.08	8
20.	72	8	SSL	5.39	8
20.	75	5	ST	6.11	8
20.	72	8	ST	5.58	8
Corn starch					
20.	75	5	SSL	6.02	8
20.	72	8	SSL	5.39	8
20.	75	5	ST	6.31	8
20.	72	8	ST	5.97	7
Potato starch					
20.	75	5	SSL	6.12	9
20.	72	8	SSL	6.00	8
20.	75	5	ST	6.55	8
20.	72	8	ST	6.28	7

Using soy flour to improve the nutritive value of high-starch bread.

Because the breads discussed lacked protein, we performed additional studies using soy flour (defatted) to raise bread's protein quantity and quality. Tables 6 and 7 give typical results.

Baking performances varied with different blends and surfactants. Of nonwheat flours and starches, corn and potato starches outperformed corn and rice flours (Table 6). EM was slightly more effective than ST or SSL in increasing loaf volume, while SSL gave the finished bread a slightly better grain than the others (Tables 6 and 7). Soy flour impaired baking quality (loaf volume) of the experimental bread more than corn starch when the same amount (80 or 75 %) of wheat flour was used in the blend for breadmaking (Table 7).

Most important, however, was that acceptable breads could be made from a blend consisting of 75 % wheat flour, 20 % nonwheat flour or starch, and 5 % soy flour by adding 0.5 % SSL, ST, or EM.

TABLE 7

Volume and grain score of breads from indicated blends of wheat flour II, corn starch, soy flour, and 0.5 % EM

Wheat flour %	Corn starch %	Soy flour %	Avg.sp.vol. cc/g	Grain score
80.	15	5	6.81	8(0)
80.	12	8	6.38	8
75.	20	5	6.78	8
75.	17	8	6.32	8
70.	25	5	6.00	6(0)

Protein and amino acid contents and chemical score of supplemented, fortified breads

To show the nutritive improvement of breads fortified with soy flours, we prepared three types of supplemented, fortified breads from a blend of wheat flour, corn flour and soy flour. 0.5 % SSL, rather than EM or SST, was used because more countries have approved it as an additive for bakery products than the others. Protein contents of supplemented breads could be raised from 13.0 % to 15.4 or 16.7 % by fortifying with 5 or 8 % soy flour, respectively. The regular bread prepared from wheat flour II under the same conditions contained 14.3 % protein. Adding 5 % soy flour more than offset the protein-lowering effect of corn flour.

In addition to the marked increase in protein content, protein's nutritive qualiy was improved, as shown by its amino acid content and chemical score (Table 8). All the essential amino acids except methionine and cystine, analyzed and expressed as mg. per g. protein, increased with soy fortification. The chemical score was calculated according to a provisional amino acid scoring pattern proposed by a recent joint FAO-WHO Expert Commitee, 1973. Chemical scores, of course, increase as amino acid content increases. From the Table, it can be seen that lysine and threonine, though their chemical scores incrase with soy fortification, were still the first and second limiting amino acids in the experimental breads. Soy protein is deficient in sulphur-containing amino acids so soy fortification reduces them slightly. Some amino acids such as lysine are not fully available after bread is baked, so the chemical score, calculated from total amino acids-not total available amino acids-tends to exaggerate the biological value of some amino acids like lysine.

Storage test

For storage evaluation, breads were made from wheat flour II and various blends, with or without 0.5 % SSL. Results, presented in Table 9, show average

TABLE 8

Protein percentage, amino acid content (mg/g protein), and chemical score of breads prepared from indicated blends of flours treated with 0.5 % SSL

Amino acid	World Health Organization Requirement pattern content mg/g	Breads: 80 % wheat fl. + 20 % corn fl. content mg/g	chemical score	75 % wheat fl. + 20 % corn fl. + 5 % soy fl. content mg/g	chemical score	72 % wheat fl. + 20 % corn fl. + 8 % soy fl. content mg/g	chemical score
Lysine.	55	20	36	25	45	28	50
Threonine.	40	30	75	33	82	34	85
Methionine and cystine.	35	34	97	32	91	31	89
Leucine.	70	77	110	76	108	79	112
Isoleucine.	40	39	97	40	100	42	105
Valine.	50	49	98	51	102	52	104
Phenylalanine and tyrosine. . . .	60	82	136	84	140	88	146
Tryptophan.	10	–	–	–	–	–	–
Protein %.		13.0		15.4		16.7	

TABLE 9

Average gelometer reading (g) of stored breads made from wheat flour and indicated blends

Bread	Days stored: No SSL 1	No SSL 3	No SSL 5	0.5 % SSL 1	0.5 % SSL 3	0.5 % SSL 5
Wheat flour.	91	151	191	72	99	142
80 % wheat flour + 20 % corn fl.. .	288	379	589	123	185	258
75 % wheat flour + 20 % corn flour + 5 % soy flour.	281	391	521	136	165	253

gelometer readings (g.) for loaves stored 1, 3, or 5 dyas. Supplemented breads firmed rapidly; their crumbs became so firm that all gelometer readings registered more than 500 g. SSL effectively helped stored breads retain softness. Breads supplemented with nonwheat flours and 0.5 % SSL compared favorably with the control breads supplemented with corn starch and SSL or ST gave similar results. No supplemented bread showed any sign of off-flavor after five days' storage.

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A study of the application of modern industrial techniques to a traditional process based on the cowpea, *vigna unguiculata* (L.) walp.

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ABSTRACT

A laboratory scale process was developed for the production of canned moinmoin product. Difficulties were encountered due to the swelling and gelling of the slurry. Studies were undertaken to ascertain the influence of solids content on the swelling and gelling properties of the slurry and the textural characteristics of the canned product.

It was found that the swelling and gelling of the slurry as well as the resistance to compression of the canned product were dependent on solids content. The cowpea was fractionated into its major components and the contribution of each to the rheological character of the processed material was studied. It was found that the carbohydrates, particularly the starch fraction, contributed most to the viscosity, while the protein reduced the viscosity of the heated slurry.

INTRODUCTION

Pulses represent an important source of available protein for human nutrition in many economically developing countries. Although, the groundnut (*Arachis hypogea*) and the cowpea are the most widely cultivated legumes in Africa (Whyte and Moir, 1959) and (Aykroyd and Doughty, 1964), the cowpea is the most widely eaten (Doughty and Orraca-Tetteh, 1966), groundnuts being grown primarily as cash-crops for export. The cowpea constitutes the major grain legume in Nigeria (Olayide et al., 1972), where it is cultivated for human consumption (Oyenuga, 1968). The production of this legume in commercial quantities in Nigeria has been reported by Sellschop (1962) and Anonymous (1966).

The importance of the cowpea as food, especially in communities where the consumption of animal products is inadequate, lies primarily in its high protein content. The mature seed is reported to contain 20-25 % (as-is or natural moisture basis) crude protein (Doughty and Orraca-Tetteh, 1966), (Oyenuga, 1968), (Dean,

1958), (Johnson and Raymond, 1964), (Kuzayli et al., 1966) and (Aykroyd and Doughty, 1964). It is commonly used in the dry, mature state for culinary purposes. One of the various forms in which the cowpea appears in the Nigerian diet is as moinmoin (also known as maimai, ole etc.). Oyenuga (1968) has described a traditional moinmoin process. A typical traditional process starts with an overnight steeping of the seeds in water, followed by decortication by hand-rubbing or saddle stone and then wet milling, usually on a saddle stone. Ingredients such as salt, onion, pepper, palm or groundnut oil, and also meat, fish, shrimps or eggs are included according to purse and taste. The slurry is mixed, with additional water if necessary, until the right consistency is attained. The mixture is finally wrapped in special leaves or poured into cans and steamed.

Moinmoin is reported by Maliki (1967) to have a high nutritional value as compared with six common traditional Nigerian dishes. The traditional process is, however, laborious and time-consuming. Moinmoin has a limited shelf life (24 hours maximum) under warm ambient conditions (25-30)° C and no evidence was found in the literature of any significant work done to apply modern food processing and preservation techniques to this product.

The broad aim of the project is to establish the feasibility of applying modern technological procedures to the traditional moinmoin process. That part of the work reported here deals with the study of the swelling and gelling behaviour of the basic raw material and some of the factors influencing the rheological characteristics of the processed product.

MATERIALS AND METHODS

Cowpeas

California black-eye cowpeas were used in the preliminary work on decortication and the swelling/gelling characteristics of the slurry. A supply of Nigerian cowpeas of the Prima variety was specially imported for the studies on the factors influencing the physical characteristics of the processed moinmoin. All other moinmoin ingredients were of normal commercial grades, purchased locally.

Physical Test Procedure

A Brabender Viscograph was used to study the rheological characteristics of the cowpea slurry. Both the heating and cooling profiles were obtained.

An empirical method was devised for studying the swelling capacity of the slurry. This term is defined as

$$\left(\frac{\Delta V}{V} \times 100\right)\%$$

where

ΔV = The difference between the maximum volume attained during heating and the original volume of cowpea slurry.

V = Original volume of slurry.

A predetermined quantity of slurry was placed in a measuring cylinder, immersed in a water bath and heated in a specified time to a maximum temperature of 98° C. No further significant expansion occured at this temperature. The increase in volume of the slurry sample was noted and used to calculate the swelling capacity, as above.

The setting temperature was determined using the apparatus shown in Figure 1. This equipment comprises a small electrically driven stirrer fitted with a speed indicator. A small permanent magnet was attached to the motor spindle. A stationary pick-up coil was mounted immediately above the magnet. The current (mA) induced in this coil was proportional to the speed of rotation of the stirrer and was used as an index of speed. The temperature at which a sudden and substantial drop in speed was noted was taken as the setting temperature of the slurry under test. A typical curve obtained with this equipment is shown in Figure 2.

The textural measurements on the processed materials were carried out using the Instron

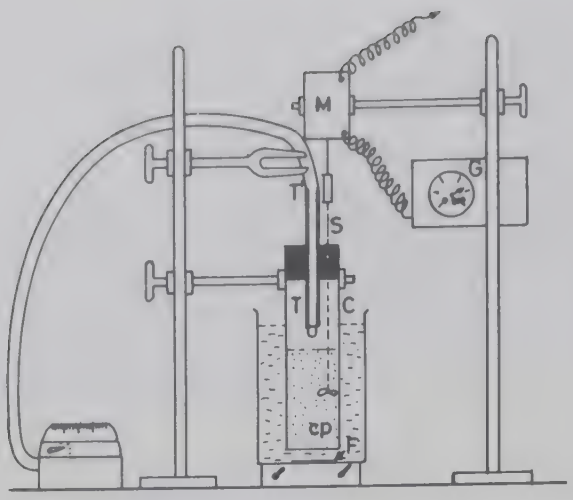


Fig. 1.–Setting temperature apparatus.

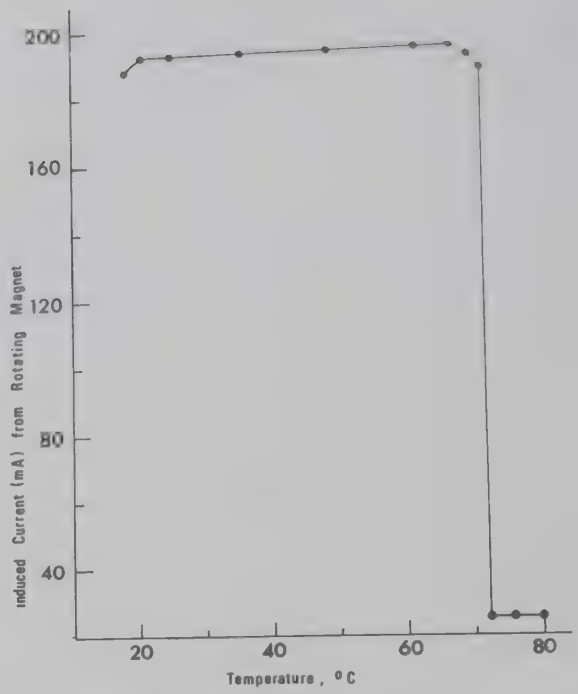


Fig. 2.–Typical curve obtained in a setting point determination.

Universal Testing Machine, the operation of which has been described by Bourne et al. (1966) and by Hindman and Burr (1949). It was installed in a room maintained at 21° C.

Cylindrical samples, 1.8 cm diameter and 1.0 cm high, were prepared and compressed by a plunger (also 1.8 cm diameter) descending vertically at 1 cm/min. The resistance offered by the sample was detected by a load cell on which it was placed. A typical Force-Distance curve obtained in such a test is shown in Figure 3. Various measurements were made from these curves and used as indices of texture. In this paper, however, only one measurement i. e. maximum force recorded, is used.

Separation of Starch and Protein Fractions

For the purposes of studying the rheological properties of the major componentes of cowpea it was necessary to isolate the starch and protein fractions.

The starch was isolated by extraction in water as described by Whistler et al. (1954). The pH of the extract was adjusted to 6.3, which is the pH of the original cowpea slurry, and dry starch prepared by freeze-drying. The residual fat (0.3 %) was extracted with n-pentane.

As a preliminary to preparing the protein fraction a defatted flour was prepared from the cowpea cotyledon. Its protein solubility profile was then determined as shown in Figure 4. For this purpose, a combination of the dye-binding using C. I. Acid Orange 12 (Romo et al. 1974) and the Kjeldahl techniques were used in determining the protein content at each pH value. Using these data, the method of protein extraction, described by Flink and Christiansen (1973), was modified and used to prepare the

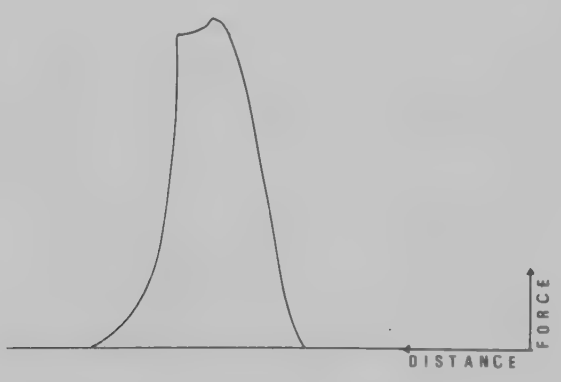


Fig. 3.–A typical force-distance curve obtained with the instron.

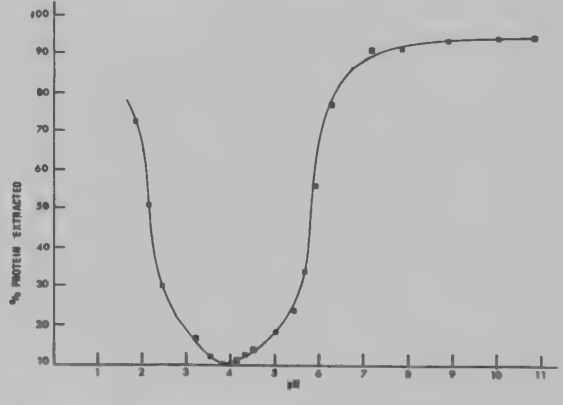


Fig. 4.–A protein solubility profile of cowpea cotyledon.

protein fraction from the cowpea cotyledon. The extraction was carried out at a pH of 10.5 and the protein precipitated at pH 3.8. The protein extract was then adjusted to a pH of 6.3 and freeze-dried.

As a bye-product of the protein extraction, a third fraction was prepared which was essentially a fat - and protein-free carbohydrate fraction. This was also adjusted to pH 6.3 and freeze-dried.

Chemical test methods.

Moisture content of the raw materials and processed product was determined by drying in an air oven at 130° C for 2 hours (A. O. A. C., 1970). Crude protein was determined by the macro-Kjeldahl method (Pearson, 1970); fat by the Soxhlet method (Pearson, 1970); crude fibre by defatting, drying, refluxing with acid and then alkali, and ashing at 600° C. (Pearson, 1970); ash by incineration at 600° C. (A. O. A. C., 1970). The total carbohydrate content was calculated by difference (Harding et al., 1965).

RESULTS AND DISCUSSION

Canning or dehydration appeared to be the two methods of preservation most likely to be applicable to moinmoin preservation.

The decision was made to concentrate initially on the development of the canned product. Among the considerations which led to this were:

a) the fact that a canned product could contain all the ingredients of a complete moinmoin dish and would require no further treatment other than warming, prior to consumption.

b) any commercial exploitation of a process for moinmoin is likely to be on a small scale initially and this could be more easily achieved by a canning operation rather than dehydration.

The stages in the production of moinmoin are illustrated in Figure 5. Sorting was done by hand and all foreign matter, unsound beans, etc. removed by hand. Washing was carried out using high pressure water sprays. The steeping stage is a necessary prerequisite to the removal of the husk. In the traditional process steeping is usually overnight. Some experiments were carried out to ascertain the optimum steeping time and the effect of temperature. Samples of sound cowpeas, consisting of known numbers of seeds, were steeped in an excess of distilled water at three different temperatures (20, 30 and 50)° C. After various time intervals, samples were removed, placed in the bowl of a domestic mixer, and agitated at constant speed for a fixed time (2 minutes). The beans were then examined and the number of satisfactorily decorticated seeds recorded.

The results are shown in Figure 6. As can be seen, at each temperature the ease of removal of the husks initially increases with increase in steeping time and that a point is reached beyond which no further improvement was noted. The effect

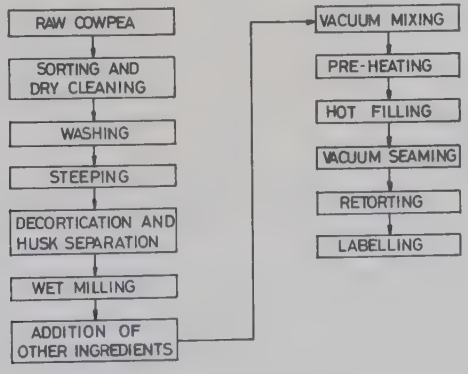


Fig. 5.–Stages in the production of canned moinmoin (laboratory scale).

of steeping depends on the temperature of the water, the higher temperatures facilitating the husk removal. It was also apparent from the turbidity of the steeping water at the higher temperatures that some leaching of solids had occured.

The nature and quantity of solids lost by soaking was not investigated. Doughty and Orraca-Tettch (1966) suggested that soaking time and changing of steeping water could help in reducing the presence of water-soluble toxins in legumes. Mustakas et al. (1967) claimed that soaking is needed for uniform cooking of beans. No loss of nutrient occurs when the seeds are steeped in their husks and the soaking water is not warmed (Aykroyd and Doughty, 1964). Dawson (1952) reported that some 25 % of the thiamine originally present in legumes may be lost due to diffusion if the seeds are put into boiling water to produce rapid softening. This aspect merits further investigation.

Results shown in Figure 7 indicate that the husk imbibes more water than the cotyledon. This confirms a similar statement made about legumes by Aykroyd and Doughty (1964). By comparing the steeping curve at 30° C in Figure 6 with Figure 7, it would seem that the optimum steeping time (about 3 hours) corresponds to the point where the cotyledon has imbibed its maximum amount of water. It could be that the swelling of the cotyledon contributed to the loosening of the husk which is already softened due to the absorbed water.

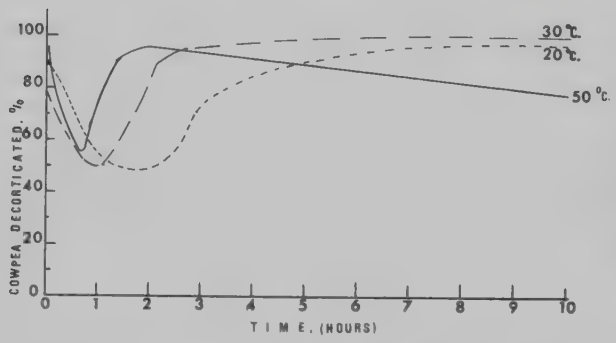


Fig. 6.–The influence of steeping time and temperature on the effectiveness of the decortication method.

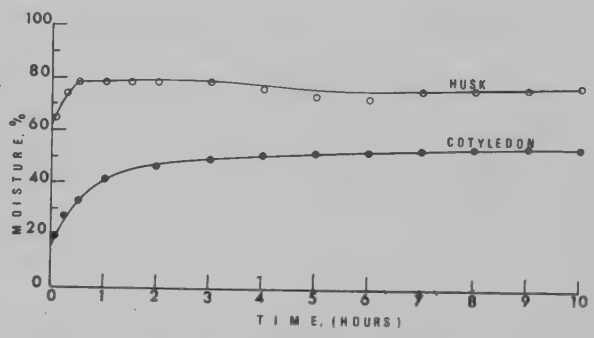


Fig. 7.–Variation in moisture content with time of cowpea husk and cotyledon during steeping in water at 30°C.

As an alternative to the use of the mixer for removing the softened husk after steeping, a device, consisting of a wooden board coated with a fine silicon carbide paper and a cylindrical roller covered with a coarse silicon carbide paper has since been used in laboratory experiments. This has proved very effective. It could be that this principle is applicable to decortication of steeped cowpeas on a commercial scale.

Some limited experiments using sodium carbonate in the steeping water (0.5 %) indicated that husk removal was made easier, a one hour steeping time being adequate. However, above 30° C a blue-black pigment, released from the cowpea eye, discoloured the cotyledon.

A single experiment, in which a batch of cowpeas was put through a commercial bean decorticator in a dry condition, showed that although this was effective, losses were high (about 10 %).

Early experience at the filling stage revealed difficulties in controlling headspace, avoiding spillage, and expansion of the can contents during processing. These difficulties arose as a result of swelling and gelling of the material. It was decided to look at these characteristics in detail. In Figure 8 the swelling capacity can be seen to dependent on solids concentration of the slurry. These data enabled a more accurate control to be exercised over the can headspace.

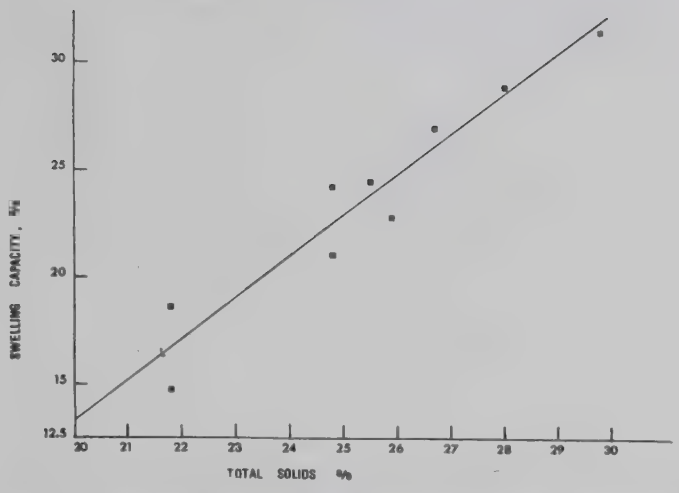


Fig. 8.–Swelling capacity Vs total solids content of cowpea slurry.

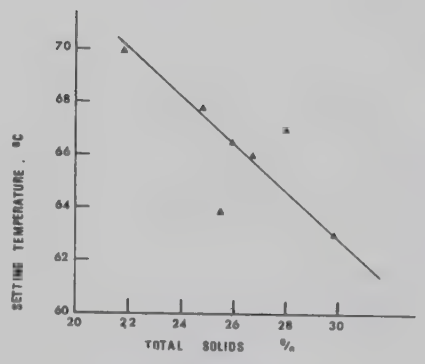


Fig. 9.–Setting temperature Vs total solids content of cowpea slurry.

The setting (gelling) point was found to be in the temperature range 63-70° C, and to be dependent on solids concentration as shown in Figure 9. This data enabled pre-heating conditions to be specified for different formulations. To reduce the air content of the slurry, mixing under vacuum, prior to pre-heating, was found to be effective. This reduced can strain during retorting, spillage problems during vacuum seaming and resulted in the texture of the final product being smoother and less porous.

The pH of the moinmoin slurry based on the model formula (Table 1) was found to be 5.6 i. e., in the category of low acid foods. The time-temperature relationship at the thermal centre of the can during processing was obtained using fine wire thermocouples. Using these data, an appropriate process was calculated. Currently, storage trials are in progress to verify the adequacy of this process.

TABLE 1

A model moinmoin formula

	Ingredient	%
1	Cowpea slurry (approx. 85 % moisture).	77.53
2	Onion (fresh, milled).	7.44
3	Groundnut oil.	6.95
4	Egg (fresh, whole).	3.72
5	Tomato puree (approx. 82 % moisture).	2.23
6	Salt (table).	1.13
7	Shrimp (dry, pulverised).	0.75
8	Pepper (Nigerian, Red)	0.25

A limited consumer survey was carried out in which all the participants were Nigerians familiar with moinmoin. The results indicated that texture was a very important quality attribute of this product. An investigation was undertaken to study the influence of variations in formulation and processing conditions on the texture of canned moinmoin. This study is still in progress and the results presented here cover only the early stages of the programme. The effect of solids concentration on the firmness of canned cowpeas slurry was studied. The results (Figure 10) show that there is alinear relationship between firmness (measured as indicated in 'methods') and total solids content. A typical proximate composition for

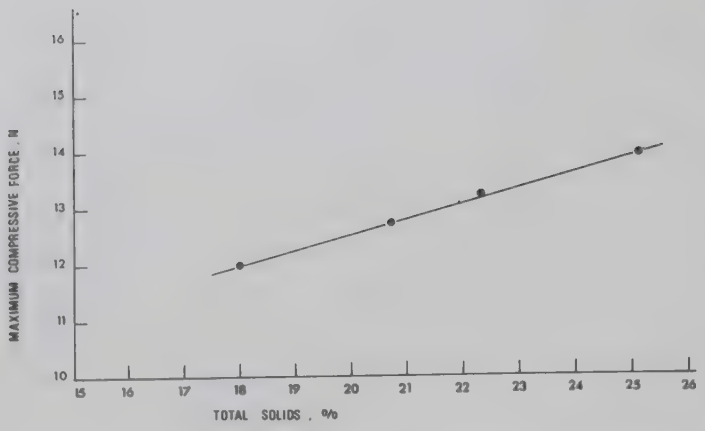


Fig. 10.–Variation of maximum compressive force with total solids content of canned cowpea slurry.

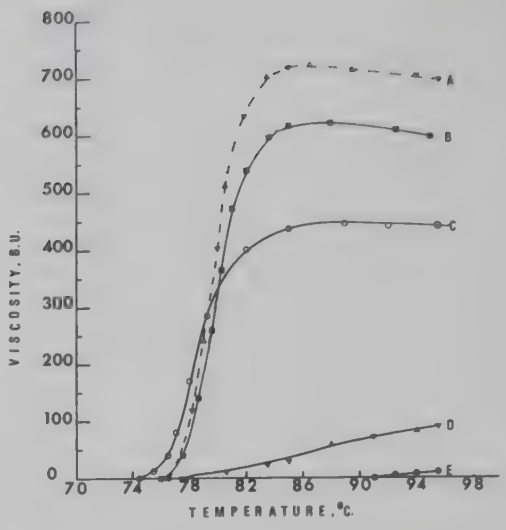


Fig. 11.–Variation in viscosity (Brabender units) with temperature of the major components of cowpea during heating.
A = 6.9 % starch only
B = 6.9 % starch + carbohydrates (50–50 mixture)
C = 6.9 % carbohydrates only
D = 6.9 % cotyledon only
E = 6.9 % protein isolate only.

whole cowpeas, cowpea cotyledon, and canned moinmoin (model formula) is shown in Table 2. It seems reasonable to assume that the major components i. e., the carbohydrates, mainly starch, protein, and fat are likely to influence the texture of the canned product. Initially, it was decided to look at the relative contributions of the carbohydrate and protein components to the texture of processed cowpea slurry. The components were separated as described in the 'methods' and their rheological behaviour studied, using the Brabender viscograph. The results (Figures 11 and 12) strongly indicate that the starch element contributes most to increasing the viscosity of the system and that the presence of other carbohydrates, e. g., fibre, reduces the viscosity. The protein element appears to be responsible for the much lower viscosity exhibited by the cotyledon slurry as compared with the protein-free carbohydrates. All the systems tested show a marked increase in viscosity on cooling but the differences between them persist.

TABLE 2

Proximate composition of whole cowpea, cowpea cotyledon, and canned moinmon (expressed as % dry matter)

Composition	Sample		
	Cowpea whole	Cowpea cotyledon	Moinmoin
1 Dry matter.	86.3	88.0	23.3
2. Crude protein.	27.1	26.4	19.8
3. Crude fat.	1.2	1.5	33.1
4. Crude fibre.	–	1.2	–
5. Total ash.	4.3	3.6	7.5
6. Total carbohydrates.	67.4	68.5	39.6

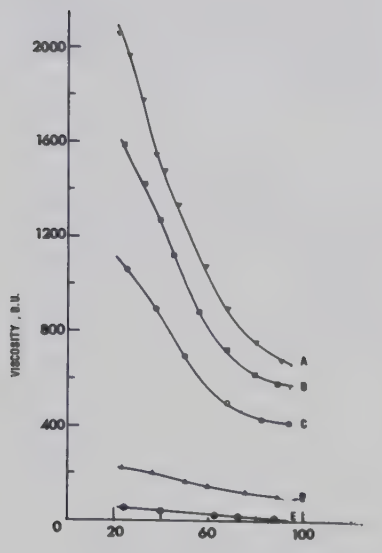


Fig. 12. Variations in viscosity (Brabender units) with temperature of the major components of cowpea on cooling.
A = 6.9 % starch only
B = 6.9 % starch + carbohydrates (50 50 mixture)
C = 6.9 % carbohydrates only
D = 6.9 % cotyledon only
E = protein isolate only.

CONCLUSION

The results of the laboratory scale experiments reported in this paper indicate that the application of a canning process to the production and preservation of moinmoin appears to be feasible. The data assembled on the gelling and swelling characteristics of the cowpeas have led to the development of a satisfactory procedure for filling and closing the cans. A heat process has been worked out for this product and storage trials, still in progress, so far indicate that this process is adequate.

The preliminary work on the factors influencing the texture of the product indicate that the texture is dependent on the solids content in the slurry and on the relative proportions of the starch, protein and fibre that make up these solids. An increase in the starch content results in a more viscous, firmer product, while an increased protein content has the opposite effect. The data obtained in these experiments should enable some control to be exercised over the texture of the final product by varying the formula, particular with respect to starch: protein ratio.

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State of the supply of raw materials for the meat industry in Poland

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ABSTRACT

The period of War-operation and occupation caused Poland, in comparison with other countries, to suffer the greatest global losses in the population of farm-animals.

At the present time Poland has 11452.5 thousand head of cattle, including 5993.2 thousand head of cows.

The joint global production of beef- and veal-cattle for slaughter alike, in absolute numbers, as well as in conversion per 100 has. of land in use, or also per statistical cow, showed a tendency for rapid growth from 498.6 to 742.2 thousand tons (growth-index 148.7). Veal-cattle for slaughter was kept at an unchanged level.

The studies of the development of the production and supply of beef- and veal-cattle for slaughter in Poland from 1960 to 1972, carried through as a composite unity, allowed the Author to become more acquaninted with the state and directions of its development.

INTRODUCTION

Specific trait of development of the socio-political relation our generation is a formation in the particular countries and regions of the World of the exact integration among the plant- and stock-production and fishery and processing and distribution of food-products (2), (Table 1, 2, 3).

The period of War-operation and occupation caused Poland, in comparison with other countries, to suffer the greatest global losses in the population of farm-animals.

The initial state in 1945, in comparison with 1939, came out, for cattle, to merely 31.5 per-cent, (Lublin-Voivod-ship-47.2 per-cent (15), Szczecin-Voivodship-2.5 per-cent (24)), while in such countries as Holland, Belgium France, Czechoslovakia, or Roumania it was from 82.4 - 93.9 per-cent (26).

OBJECTIVES

At the present time Poland has 11452.5 thousand head of cattle, including 5993.2 thousand head of cows (Table 4), of which 73.0 per-cent of the cattle-population belongs to the black-and-white lowland

TABLE 1

Average consumption of some articles per inhabitant in Poland

Name of article	1960	1970	Index Year 1960 = 100	1972	Index Year 1960 = 100
Grain-products.	145.0	131.0	90.3	127.0	87.6
Potatoes.	223.0	190.0	84.7	187.0	83.8
Meat.	42.5	53.0	123.7	59.3	139.5
Fats.	13.6	18.0	122.3	18.3	134.5
Milk.	352.0	401.0	113.9	410.0	116.4
Eggs.	143.0	186.0	130.0	196.0	137.0

Source: GUS - 1973 - Rocznik Statystyczny - 1973; My own calculations, (12).

TABLE 2

Meat-consumption in selected countries in the years 1961-1963. (Annual average, in Kgs., per inhabitant, according to FAO) (22)

Countries		Meat-gross	Beef and veal	Pork	Poultry	Mutton	Other meats
Poland.	kgs	44.3	8.7	28.5	1.5	0.7	4.9
	per-cent	100.0	19.6	64.3	3.4	1.6	11.1
German Democratic Republic.	kgs	55.3	13.1	31.8	4.5	–	5.9
	per-cent	100.0	23.7	57.5	8.1	–	10.7
Union of Soviet Socialist Republic.	kgs	37.5	12.2	12.4	3.6	3.9	5.4
	per-cent	100.0	32.5	33.1	9.6	10.4	14.4
German Federal. Republic.	kgs	60.5	20.2	30.8	5.0	0.3	4.2
	per-cent	100.0	33.4	50.9	8.3	0.5	6.9
France.	kgs	85.9	31.1	25.6	8.6	3.0	17.9
	per-cent	100.0	36.2	29.8	9.7	3.5	20.8
Italy.	kgs	30.7	14.8	6.8	4.4	0.8	3.9
	per-cent	100.0	48.2	22.2	14.3	2.6	12.7

TABLE 3

Consumption of meat in Poland (39)

Kinds of meat	1965	1970	1990
Yearly per inhabitant of meat and animal fat - in kgs.	56.0	61.3	92.5-102.5
Percentage:			
–Beef and veal.	23.0	27.5	29.5- 32.6
–Pork.	69.4	62.4	53.5- 51.7
–Mutton.	1.0	1.1	1.3- 1.2
–Poultry.	4.7	6.2	12.3- 11.0
–Other meats.	1.9	2.8	3.4- 3.5

TABLE 4

Cattle and the cow-population in 1960-1972

Years	Cattle in thousands of head	Indices		Cows in thousands of head	Indices		Percentage of cows
		Annually progressive	Year 1960 = 100		Annually progressive	Year 1960 = 100	
1960	8695.1	–	100.0	5884.7	–	100.0	67.7
1961	9168.1	105.2	105.2	5915.4	100.5	100.5	64.6
1962	9589.5	104.6	110.3	6022.4	101.8	102.3	62.8
1963	9841.4	102.6	113.2	6070.3	100.8	103.1	61.7
1964	9939.7	101.1	114.3	6013.1	99.1	102.2	60.5
1965	9947.2	100.1	114.4	5920.3	98.7	100.6	59.4
1966	10390.8	104.4	119.5	6013.6	101.7	102.2	57.9
1967	10767.5	103.6	123.8	6142.5	102.1	104.4	57.5
1968	10940.0	101.5	125.8	6193.8	100.8	105.2	56.6
1069	11048.9	101.0	127.7	6250.2	100.9	106.2	56.5
1970	10843.5	98.1	124.7	6081.8	97.2	103.3	56.1
1971	11076.3	102.3	127.4	6041.0	99.3	102.6	54.5
1972	11452.5	103.4	131.6	5993.2	99.2	101.3	52.3

Source: GUS - 1973 - Rocznik Statystyczny - 1973; My own calculations, (12).

breed, 5.5 per-cent to the red-and-white lowland breed, 17.5 per-cent to the Polish-red breed, 0.5 per-cent to the simmentaler-breed, and 3.5 per-cent is made up of non-breed cattle and the hybrids (26).

Within the range of the period analysed, the several recuring changes in the breed regionalization were accomplished. At the present moment, the projects for the development of cattle-production require the fastest possible verification of the breed-regionalization which has been in force up to present —taking the productive— types of cattle into consideration. It is imperative, likevise, that a decision concerning the localization of milk and beef-cattle production-regions be made (6, 7, 4, 5, 1, 2, 3, 32, 35).

The stock of cattle per 100 has. of land in use comes out 59.1 head of cattle, including cows - 30.9. The difference between the marginal-voivodships, to: Rzeszów - 77.1, Bialystok - 47.3 head of cattle - 100 - 61 (12). A still greater difference exists in the stock of cows - Kraków - 54.0, Szczecin - 19.7 head of cows - 100 - 36 (12).

The final stage of stock-production on the farm - is normally sale (2, 1, 20, 28, 29, 38) (Tabie 5, 6). Recently, a nearly twofold increase in the tonnage of beef-cattle purchased for slaughter; that is, from 408,9 thousand tons in 1960 to 706.7 thousand tons in 1972, that is - growth-index 172'8 (Table 7).

The supply of calves was formed comewhat differntly; which till came out to 50 per-cent of the positions of 1960. This was a result of unfavourable prices for the calves for slaughter, and therefore the cottage-slaughter of calves increased. From 1968 a marked rise in the prices of calves for slaughter occured, for both the free-marked purchasing and the compulsory supplies (Table 5, 6, 7, 8, 9, 10, 11, 12, 13, 14) (1, 3, 4, 5, 16, 19, 27, 30, 34, 35, 36, 37, 38).

The period under study slowed serious fluctuations in purchasing regarding the fodder-situations, the price-relations, and so on. That is why, in general, cattle-purchasing develops faster than does the cattle-population, when the sale of calves is limited simultaneously (Table 5, 6, 7, 10) (21, 33).

The demonstrated proportions fo purchasing do not, however, comprise the total production destined for sale on the marked, because, in addition to the basic home-market, the export market has increased dynamically in recent years. This market is comprised of only two categories of slaughter-cattle; these are: bulls "HEAVY (over 420 kg) and young-bulls "LIGHT-WEIGHT" (of an average weight of about 200-240 kg), as well as being comprised of usable-cattle (Table 9) (6, 7, 15, 18, 20, 23, 24, 25, 26, 27, 29, 31, 32, 34, 35, 37, 38).

In the analyses conducted on the development of purchasing, a great dependence of the

purchasing of the marketable production of beef- and veal-cattle for slaughter on the development of the cattle-population was found. The fact that the developing-tendency of the cattle-population was outstripped by the purchase of beef- and veal-cattle for slaughter was a result of changes in the herd-structure, and in the proportions between the number of fattened beef-cattle and calves- to the profit of the cattle. There was also a rise in the average weight of the head of the cattle sold (Table 11). The fluctuations occurring in both the production and the animals designated for slaughter in the various seasons of the year are above all bound up with the seasonal changes in the fodder-resources and

TABLE 5

Relation of the prices of cattle for slaughter from the contracted and optional-deliveries to the prices of the fundamental fodders in Poland in 1960-1972

Years	1 kg of the cattle contracted for slaughter figure out to an equivalent of kgs of:				1 kg of optional deliveries of the cattle for slaughter figures out to an equivalent of kgs of:			
	Rye	Barley	Potatoes	Hay	Rye	Barley	Potatoes	Hay
1960	5.1	4.7	11.0	12.1	3.9	3.6	9.2	9.3
1965	4.2	3.9	12.3	8.8	3.1	2.9	9.3	6.6
1970	4.1	3.9	12.6	10.8	2.9	3.2	10.2	8.7
1971	4.5	4.2	11.8	12.4	3.4	3.2	8.8	9.3
1973	5.1	4.7	12.1	14.0	3.8	3.5	9.0	10.4
Arithmetical mean	4.6	4.3	11.9	11.6	3.4	3.3	9.3	8.8

Source: GUS - 1973 - Rocznik Statystuczny - 1973; My own calculations, (12)

TABLE 6

The ratio of the prices of the beef-cattle for slaughter from contract-deliveries, and from optional annual supplies of live-stock to the State, to the veal-cattle for slaughter and to milk, as well as the ratio of the prices of the veal-cattle for slaughter from optional-deliveries to the milk in Poland in 1960-1972

Years	1 kg of beef-cattle for slaughter (from contract) comes out to:		1 kg of beef-cattle for slaughter (from optional-deliveries) comes out to:		1 kg of veal-cattle for slaughter (from optional-deliveries) = litres of milk
	Kgs of veal-cattle for slaughter (from optional-deliveries)	Litres of milk	Kgs of veal-cattle for slaughter (from optional-deliveries)	Litres of milk	
1960	1.13	6.02	0.87	4.62	5.31
1965	0.93	5.65	0.69	4.20	6.06
1970	0.86	5.70	0.69	4.62	6.66
1971	0.89	5.46	0.67	4.09	6.09
1972	0.94	5.31	0.69	3.96	5.65
Arithmetical mean	0.95	5.63	0.72	4.29	5.95

Source: GUS - 1973 - Rocznik Statystyczny - 1973; My own calculations, (12)

TABLE 7

Development of the purchasing of beef- and veal-cattle for slaughter between 1960-1972 (In tonnage)

Years	Beef-cattle for slaughter			Veal-cattle for slaughter			Beef- and veal-cattle for slaughter		
	In thousands of tons	Annually progressive index	Year 1960 = 100	In thousands of tons	Annually progressive index	Year 1960 = 100	In thousands of tons	Annually progressive index	Year 1960 = 100
1960	408.9	–	100.0	89.7	–	100.0	498.6	–	100.0
1965	594.6	145.3	145.3	49.7	55.2	55.2	644.3	129.4	129.4
1966	601.6	101.0	145.8	45.7	91.4	50.8	647.3	100.5	129.7
1967	716.0	119.0	175.0	45.0	98.0	50.0	761.0	117.5	152.5
1968	761.0	106.3	186.0	50.8	112.4	56.4	811.8	106.6	162.7
1969	848.2	111.4	207.3	65.9	130.4	73.2	918.1	113.0	183.9
1970	770.8	90.9	188.4	54.0	82.2	60.0	824.8	89.8	165.1
1971	748.5	97.1	183.0	54.8	101.5	60.9	803.3	97.4	160.9
1972	706.7	94.5	172.8	35.5	64.6	39.4	742.2	92.4	148.7

Source: GUS - 1973 - Rocznik Statystyczny - 1973; My own calculations, (12).

TABLE 8

Development of the production of the beef- and veal-cattle for slaughter (In thousands of head of cattle)

Years	Cattle			Calves		
	In thousands of head of cattle (without calves)	Annually progressive index	Year 1960 100	In thousands of head of calves	Annually progressive index	Year 1960 = 100
1960	1344.1	–	100.0	3073.6	–	100.0
1965	2101.2	156.3	156.3	2365.7	77.0	77.0
1966	2056.0	97.8	153.0	2335.3	98.7	76.0
1967	2318.2	112.7	172.5	2294.7	98.2	74.6
1968	2585.1	111.5	192.3	2377.2	103.6	77.3
1969	2796.2	108.2	208.0	2578.3	108.4	83.8
1970	2626.5	94.0	197.6	2070.2	80.3	67.3
1971	2593.9	98.7	193.0	2141.4	103.4	69.7
1972	2420.9	93.3	180.1	1832.6	90.7	59.6

Source: GUS - 1973 - Rocznik Statystyczny - 1973; My own calculations, (12).

the fodder-balance of the agriculture, the seasonal formation of the price-relations of the fodder to the animal for slaughter, the varying intensity of farm-work at different times of the years (1, 2, 4, 15, 18, 20, 21, 23, 27, 30, 31, 33, 36).

Outside of centralized purchasing discussed slaughter out of necessity and cottage-slaughter take place, covering to a certain degree the requirements of the farm-family for meat and its products, as also destined for bazaar-sale (9, 11, 12, 15, 23, 28, 29).

Although the balancing-method of formulating the development of the cattle-population has not yet been finalized, and is often ommited in the considerations, as a recapitulations of the present analysis

it undertook the test of construction for the National Cattle-Population for the years 1959/1960 to 1971/1972. (Table 15, 16, 17) (23, 32).

The unit-yield of milk per cow is still low; in 1972 it was a level of 2563 litres on the home-scale, and 78.9 thousand litres per 100 has. of land in use (12).

The joint global production of beef- and veal-cattle for slaughter and beef and veal alike in absolute numbers, as well as in conversion per 100 has. of land in use (from 3.3 to 5.0 tons -beef- and veal-cattle for slaughter, and from 1.7 to 2.6 tons beef and veal), of also per statistical cow (from 108.6 to 160.5 kgs. beef- and veal-cattle for slaughter, and 56.7 to 83.5 kgs. beef and veal) showed a tendency for rapid growht - from 481.6 - 863.2 thousands tons of beef-cattle for slaughter, and 235.5 to 439.1 thousands tons beef (veal-cattle for slaughter was kept at an unchanged level). (Table 17).

RESULTS AND DISCUSSION

It is past nearly 30 years a post-War work on the development of cattle-rearing in the Country. In the same time one marks that agricultural policy in Poland in the last years is developed against a background of food-policy, determining and integrated complex of the agricultural-industrial questions (31).

The studies of the development of the production and supply of beef- and veal-cattle for slaughter in Poland from 1960 to 1972, carried through as a composite unity, allowed the Author to become more acquainted with the state and directions of its development.

TABLE 9

Development of the export of the usable-cattle in Poland (In thousands of head of cattle)

Years	Thousands of head of cattle	Annually progressive index	Year 1960 = 100
1960	–	–	–
1965	29.0	–	–
1966	43.0	148.2	–
1967	87.0	202.3	–
1968	105.0	120.6	–
1969	150.0	142.8	–
1970	182.0	121.3	–
1971	237.0	130.3	–
1972	290.0	122.3	–

Source: GUS - 1973 - Rocznik Statystyczny - 1973; My own calculations, (12)

TABLE 10

Purchasing of beef- and veal-cattle for slaughter per 100 has. of land in use in 1960-1972

Years	In tonnage								
	1960	1965	1966	1967	1968	1969	1970	1971	1972
Beef and veal-cattle for slaughter.	2.5	3.3	3.5	4.1	4.5	4.8	4.6	4.6	4.4
Index - the year 1960 = 100.	100.0	132.0	140.0	164.0	180.0	191.0	184.0	184.0	176.0

Source: GUS - 1972 and 1973 - Rocznyk Statystyczny - 1972 and 1973; My own calculations, (11,12).

TABLE 11

Average weight at slaughter of 1 head of cattle and calves under veterinary-supervision in 1960-1972

Years	In kilogrammes	
	Cattle (excluding calves)	Calves
1960	338	48
1965	325	55
1966	334	59
1967	334	60
1968	336	60
1969	338	61
1970	351	72
1971	346	68
1972	356	54

Source: GUS - 1971 - Rocznik Statystyczny Rolnictwa - 1971; GUS - 1972 and 1973 - Rocznik Statystyczny - 1972 and 1973; Data from 1970-1972 - My own calculations, (10,11,12).

TABLE 12

Average dressed-yield of the cattle and calves obtained in the Meat-Industry in Poland in 1960-1972

Years	Percentage	
	Cattle (without calves)	Calves
1960	48.89	61.99
1965	49.83	62.10
1966	50.15	61.62
1967	50.05	59.67
1968	50.19	60.50
1969	50.14	60.96
1970	50.26	60.39
1971	50.55	60.33
1972	50.87	62.18

Source: GUS - 1973 - Rocznik Statystyczny - 1973; My own calculations, (12).

The contract of young-beef cattle, brought into effect in 1958, brought about a progessive change in the production-direction (from a milk to a milk-beef direction).

The period of the analysis concerns the last decade, which was characterized by an adequate arrangement of economic conditions.

— In the post-War period, the development of the cattle-population underwent certain fluctuations - in both proportions and structure. These fluctuations were a result of the change in the socio-economic conditions of the development of agriculture and prices in the animal-products. The fundamental factor limiting the development of cattle-population, however,

was the fodder-base (2, 13, 17, 18, 21, 23, 28, 29, 31, 37, 38).

— The economic-policy of the State concerning the turnover of the cattle for slaughter intensified the increase in the yield of marketable stock-production together with changes in the structure of the deliveries:

- ★ the share of the contract-deliveries was increased by simultaneously limiting the optional annual supplies o live-stock;
- ★ in the type-structure of purchasing the percentage of the cows' share decreased, and that of the heifers and steers increased;
- ★ in the frame-work of the fundamental categories of the young-beef cattle, that is - heifers and steers, a favourable increase in the share of the steers follows;
- ★ an improvement in the quality of the cattle for slaughter can be observed by increasing the percentage share- the fleshy-class - at the cost of a decrease in the shares of the small fleshy-class, fleshy-fattened class and rejected-cattle;
- ★ in the calves, heifers, cows, young-bulls - purchasing came after a favourable improvement in the average weight of the head delivered. It fell in bulls and young-cattle;
- ★ next, a serious appeasement in the seasonal character of the delivered cattle came, but it was mainly a result of the periodical differentiation in the purchasing-prices;
- ★ a favourable improvement of the slaughter-yield index of the cattle and calves followed, which should be connected with a change in the supply-structure, a change to cattle of the black-and-white lowland and red-and-white lowland breeds, and the improvement in the slaughter quality of the cattle designated for slaughter (23).
- ★ The purchasing-index in the calculation was employed - conversions, and also the production of cattle for slaughter per statistical cow. (In theory it may fluctuate from 50 to 300 kg).

TABLE 13

Total slaughtering of the cattle for 1960-1972

Years	In thousands of head			In thousands of tons of live weight		
	Cattle (without calves)	Annually progressive index	Index Year 1960 = 100	Cattle (without calves)	Annually progressive index	Index Year 1960 = 100
1960	1344.1	–	100.0	481.6	–	100.0
1965	2101.2	156.2	156.2	692.3	141.7	141.7
1966	2056.0	97.8	152.9	705.3	101.9	146.5
1967	2318.2	112.7	172.5	822.7	116.6	170.8
1968	2585.1	111.5	192.3	902.4	109.7	187.3
1969	2796.2	108.2	208.0	960.1	106.4	199.3
1970	2626.5	93.9	195.4	924.4	94.2	191.8
1971	2593.9	98.7	193.0	901.9	97.5	187.1
1972	2420.9	93.3	180.1	863.2	94.5	179.1

Source: GUS - 1973 - Rocznik Statystyczny - 1973; My own calculations, (12). (Under veterinary supervision and without - the export of live-head) (For the slaughter outside of veterinary supervision - estimated data).

TABLE 14

Total slaughtering of the calves for 1960-1972

Years	In thousands of head			In thousands of tons of live weight		
	Calves	Annually progressive index	Index year 1960 = 100	Calves	Annually progressive index	Index year 1960 = 100
1960	3073.6	–	100.0	158.4	–	100.0
1965	2365.7	76.9	76.9	142.5	90.2	90.2
1966	2335.3	98.7	75.9	139.4	97.7	88.2
1967	2294.7	98.3	74.6	145.3	103.5	91.9
1968	2377.2	103.6	77.3	147.1	101.2	93.1
1969	2578.3	108.4	83.8	159.6	108.5	100.8
1970	2070.2	80.3	67.3	136.1	85.1	85.1
1971	2141.4	103.4	69.6	121.0	89.0	76.0
1972	1832.6	85.6	59.6	98.9	81.7	61.8

Source: GUS - 1973 - Rocznik Statystyczny - 1973; My own calculations, (12). (Under veterinary supervision and without - the export of live-head.) (For the slaughter outside of veterinary supervision - estimated data.)

It seems not right the rank:

a) with the one trade-group the heifers and steers, because they represent the differentiated stock for slaughter both on score the slaughter-yield and the varied tendency to fat-accumulation;
b) the young males, which deliver the beef of high culinary value on reason of weight and not service, to category of bulls.

One should in the Polish Industry-nomenclature separate these both groups of animals in after slaughter and dressed (without bones) classification of the carcasses alike.

— The existing initial reserve for the production of young beef-cattle at a hight of approximately 1.9 million head of calves, which at slaughter in 1971 weighed 40 to 60 kg. (26). This should encourage the develoment of cooperation among the peasant, state- and cooperative- farms, as well as among the individual voivodships in the country.

— The investment-credit policy should be simplified for the small farmers as a guarantee of faster development in stock production - including that of beef-cattle. Also without significance is the maintenance of proper price-ratios between: product - fodder and product - product.

CONCLUSIONS

As a conclusion of these studies it was stated that Poland has potential reserves at its disposal, which it would be possible to mobilize by:

★ proper price-policies and their ratios:
★ a more active role for the Meat-Industry Works in the sphere of production;
★ improvement of the fodder-base by drainage and better use of the perennial green-lands;

TABLE 15

Balance of the turnover of the cattle in the years 1959-1960/1971-1972 (Part I)

Specifications	In thousands of head								
	1959-1960	1964-1965	1965-1966	1966-1967	1967-1968	1968-1969	1969-1970	1970-1971	1971-1972
1. State of the cattle-population on 30/VI.	8695.1	9947.2	10390.8	10767.5	10940.0	11048.9	10843.5	11076.3	11452.5
Including:									
a) cows.	5884.7	5920.3	6013.6	6142.5	6193.8	6250.2	6081.8	6041.0	5993.2
b) cows + 1/3 heifers over 1 year of age.	6176.1	6285.0	6405.7	6550.2	6600.7	6647.0	6470.7	6425.7	6403.8
2. Slaughter of calves.	3073.6	2365.7	2335.3	2294.7	2377.2	2578.3	2070.2	2141.4	1832.6
3. Slaughter of cattle in the coming economic year.	1491.1	2056.0	2318.2	2585.1	2796.2	2626.5	2593.9	2420.9	x
4. Export of usable-cattle.	–	29.0	43.0	87.0	105.0	150.0	182.0	237.0	290.0
5. Total (2-4).	4564.7	5450.7	4696.5	4866.8	5278.4	5354.8	4846.1	4799.3	x
6. Import of usable-head.	–	–	–	–	–	–	–	–	–
7. Decline in the number of head during the year (5-6).	4564.7	5450.7	4696.5	4866.8	5278.4	5354.8	4846.1	4799.3	x
8. Expansion (+), and decline (–) of the cattle-population during the year:									
a) under of 1 year age (in given economic-year).	x	x	+212.0	+184.0	+40.1	+78.1	+4.2	+240.9	+287.1
b) over of 1 year of age (in the coming economic-year).	x	+138.3	+63.8	+81.1	–25.6	–41.2	+32.7	+136.9	x

TABLE 16

Balance of the turnover of the cattle in the years 1959-1960/1971-1972 (Part II)

Specifications	1959-1960	1964-1965	1965-1966	1966-1967	1967-1968	1968-1969	1969-1970	1970-1971	1971-1972
9. Estimated number of the calves raised in a given economic-year (7-8) (in thousands of head).	x	x	4972.3	5131.9	52.92.9	5391.7	4883.0	5177.1	x
10. Estimated percentage of the calves raised to:									
a) the state of cows at the beginning of the year.	x	x	82.7	83.5	85.4	86.2	80.3	85.7	x
b) state of cows + 1/3 of the heifers over 1 year of age. .	x	x	77.6	78.3	80.2	81.1	75.5	80.6	x
11. Productivity of heard in percentage (7 X 100).	52.5	54.8	45.2	45.3	48.3	48.5	44.7	43.3	x
12. Estimated percentage of the slaughtered to number of calves raised.	x	x	46.9	44.7	44.9	47.8	42.4	41.3	x

Source for part I and II: GUS - 1966 - Rolniczy Rocznik Statystyczny - 1945 - 1965; GUS - 1971 - Rocznik Statystyczny Rolnictwa - 1971; GUS - 1972 and 1973 - Rocznik Statystyczny - 1972 and 1973. My own calculations, (9,10,11,12).

TABLE 17

Global-production of beef- and veal-cattle for slaughter and beef and veal (second position) in years 1959-1960/1971-1972

Specifications	In thousands of tons								
	1959-1960	1964-1965	1965-1966	1966-1967	1967-1968	1968-1969	1969-1970	1970-1971	1971-1972
1. Beef-cattle for slaughter -and beef.	481.6- -235.5	692.3- -345.0	705.3- -353.7	822.7- -411.8	902.4- -452.9	960.1- -481.4	924.4- -464.6	901.9- -455.9	863.2- -439.1
2. Increasing +, or decreasing –, weight of cattle-population.	–	–	22.3	317.7	130.0	74.8	100.0	4.8	43.7
3. Veal-cattle for slaughter -and veal.	158.6- -98.2	142.5- -88.5	139.4- -85.9	145.3- -86.7	147.1- -89.0	159.6- -97.3	136.1- -82.2	121.0- -73.0	98.9- -61.5
4. Increasing +, or decreasing –, weight of calf-population.	–	–	14.3	9.3	3.3	19.8	5.1	28.1	16.1
5. Total-cattle for slaughter -and beef and veal-together.	640.0- -333.7	834.8- -433.5	844.7- -439.6	968.0- -498.5	1049.5- -541.9	1119.7- -578.7	1060.5- -546.8	1022.9- -528.9	962.1- -500.6
6. Per 100 has of land in use -tons.	3.3- -1.7	4.3- -2.2	4.3- -2.3	5.0- -2.5	5.4- -2.8	5.7- -2.9	5.5- -2.8	5.3- -2.7	5.0- -2.6
7. Per statistical cow -kgs.	108.6- -56.7	141.0- -73.2	140.5- -73.1	157.6- -81.2	169.4- -87.5	179.1- -92.3	174.4- -89.8	169.3- -87.6	160.5- -83.5

Source: GUS - 1966 - Rolniczy Rocznik Statystyczny - 1945 - 1965; GUS - 1971 - Rocznik Statystyczny Rolnictwa - 1971; GUS - 1972 and 1973 - Rocznik Statystyczny - 1972 and 1973. My own calculations, (9,10,11,12).

★ perfection of the nomenclature of the Polish Meat-Industry, and of the classification and collection of the animals for slaughter, from farms and elsewhere.

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Variaciones estacionales en la composición de porción comestible y "semilla" de la *Rosa Aff. rubiginosa* L.

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ABSTRACT

Edible portion and seed of the Rosa aff. rubiginosa L. were studied from the viewpoint of their seasonal changes that take place in their development, ripeness and overripeness.

Humidity, protein content, ashes, ether extract, free ascorbic acid and total vitamin C were determined every fifteen days from January to June 1973.

Samples were collected in the province of Ñuble, Chile (36°45'S, 72°20'W).

Humidity and protein content of the edible portion tended to decrease through the ripeness and overripeness while ashes and ether extract remained constant. On the other hand free and total ascorbic acid increase with ripeness but then decrease in the overripeness period.

The studies carried out on the seed did not show any particular interest from the alimentary point of view.

INTRODUCCIÓN

En los últimos dos años, la *Rosa aff. rubiginosa L.* (mosqueta o coral), ha adquirido gran importancia en nuestro medio, tanto por el interés de países europeos de importar cascarilla deshidratada de esta rosácea, como la atención que han fijado en este fruto los industriales chilenos de mermeladas, jaleas, néctares, etc.

Es de destacar que en nuestro país, la mosqueta constituye realmente una maleza, a cuya eliminación se ha dedicado grandes esfuerzos, maleza que en la actualidad ha pasado a constituír una materia prima de alto valor.

Más importante aún es este interés, debido a que para realizar la recolección, despepitado y deshidratado, se necesita una abundante mano de obra, lo que ha dado la oportunidad a campesinos de escasos ingresos, procurarse un medio de subsistencia con el que no contaba anteriormente.

Es sabido que la mosqueta se consume en grandes cantidades en los países nórdicos y también por extranjeros de estos orígenes radicados en nuestro país, ya sea por las características organolépticas que comunica a mermeladas, jaleas,

bebidas, sopas, etc., como por su alto contenido en Vitamina C. Ya durante el período de la II Guerra Mundial, investigadores alemanes determinaron la importancia de la mosqueta, al encontrar 500 mg. de Vitamina C por kg. en muestras de frutos silvestres (Berger, 1952).

En Chile, la mosqueta crece expontáneamente desde la provincia de Talca hasta la de Llanquihue, encontrándose en mayor abundancia entre la cordillera de la Costa y la de los Andes.

En lo que respecta a la Región del Bío-Bío, desde el río Perquilauquén (36°16'5), hasta Victoria (38°13'5), hay unas 15.000 hectáreas cubiertas con mosqueta con una producción promedio de cuatrocientos kilos por hectárea.

En la temporada marzo/mayo de 1973 se recolectó alrededor de 1.400 toneladas de fruto, las que luego de deshidratadas y despepitadas, produjeron 250 toneladas de cascarilla (porción comestible).

La demanda de cáscara de mosqueta deshidratada en lo que se refiere al mercado nacional (mermeladas, néctares, jugos, jaleas), se estima superior a las mil toneladas anuales (Raffo, 1973).

Debido a que no se tiene antecedentes que se haya realizado estudios, ni se conozca la composición química del fruto producido en nuestro país, es que nos ha interesado estudiar tanto la porción comestible, como sus semillas, desde el momento en que se produce su completo desarrollo, hasta la época en que por efecto de la sobremaduración y/o cambios climáticos, éste comienza a caer de la planta.

Especial preocupación se ha dado al contenido, tanto en ácido ascórbico, como en Vitamina C total.

Para los efectos del presente estudio, se ha llamado "fruto" al fruto compuesto (cinorrodón) que envuelve numerosos frutos uniseminados, a los que hemos denominado "semillas" (Marticorena, 1973). La porción comestible corresponde al 49 % del fruto, expresado como producto total.

PARTE EXPERIMENTAL

TOMA Y PREPARACIÓN DE LAS MUESTRAS.

Como una forma de tener una muestra representativa del producto que en estos momentos se está recolectando para su industrialización, se tomó porciones de mosqueta que constituyeron la muestra de trabajo en una zona reducida de la Provincia de Nuble (36°45' S. 72°20' W).

En cada oportunidad se tomó al azar 3 muestras de 500 g. de frutos, cada una de ellas constituídas por alí-cuotas de 10 plantas.

Las muestras se trabajaron inmediatamente de ser recolectadas y se las preparó en la siguiente forma:

a) eliminación de restos de planta, hojas, pedúnculo, restos de infloración, etc.
b) separación de porción comestible de las semillas, teniendo especial cuidado que éstas queden libres de restos de cascarilla, especialmente en las muestras que se presentaron reblandecidas y húmedas. La cascarilla se trozó finamente y la semilla se trituró en un en un molinillo de granos.

ESTADO DE DESARROLLO DEL FRUTO

Los frutos se comenzaron a recolectar una vez que alcanzaron su pleno desarrollo, pero sin presentar signos de maduración. Las dimensiones fluctuaron entre: largo, 1.5 a 2.0 cm; diámetro, 1.0 a 1.5 cm.

Fecha	Color	Consistencia
2-I-73	Verde claro	Firme
17-I-73	Verde claro y ejemplares con zonas anaranjadas	Firme
28-I-73	Verde claro, con zonas anaranjadas; sólo algunos ejemplares verde claro	Firme
13-II-73	Ejemplares anaranjados con sólo algunas zonas verde claro	Ligeramente blando
3-III-73	Ejemplares anaranjados y rojos	Ligeramente blando
18-III-73	Totalidad de los ejemplares rojos (maduros)	Blando normal (maduro)
4-IV-73	Frutos maduros	Blando normal
23-IV-73	Frutos maduros	Blando normal
8-V-73	Frutos sobremaduros, rojo oscuro	Reblandecido; aspecto rugoso. Difícil separar semilla. No aptos para consumo
11-VI-73	Frutos sobremaduros, rojo oscuro; signos de fermentación	Reblandecidos. Arrugados. Muy difícil separar semilla. No aptos para consumo

MÉTODOS ANALÍTICOS

Determinación de humedad: secado a 100-105° C hasta peso constante.
Determinación de proteínas (N x 5,7): método Kjeldahl.
Cenizas: calcinación a 580° C hasta cenizas albas.
Extracto etéreo: método Soxhlet (Schmidt-Hebbel, 1966).
Acido ascórbico libre: titulación con 2.6 diclorofenol-indo fenol (Schmidt-Hebbel, 1958).
Vitamina C total: espectrofotometría a 520-525 nm, previa condensación con 2-4 dinitrofenilhidrazina (Strobecker, 1965).

RESULTADOS Y DISCUSION

Los resultados obtenidos en este trabajo, sobre la composición química de los productos en estudio, se expresan y comentan, por separado, referidos a peso total y referidos a materia seca (Harris, L. E., 1970).

Del estudio de la Tabla n.° 1, se observa que:

a) la humedad se va modificando en las distintas muestras, con una tendencia a la disminución a medida que se produce la maduración y sobremaduración. Sólo se produce una ruptura en estas caída en la muestra del 8.V.73.

b) las proteínas presentan valores absolutos bajos; notándose también una tendencia a la caída a medida que se llega a la sobremaduración. Los valores más bajos se encuentran en las muestras del 4.IV. y 18.V; y los más altos, en el mes de enero.

c) las cenizas y extracto etéreo, presentan variaciones pequeñas. En ambos casos, se tienen valores mínimos en el mes de abril.

d) el ácido ascórbico aumenta gradualmente, alcanzando su valor mínimo en los meses de abril y mayo (844-841 mg %), para luego caer bruscamente en el

TABLA 1

Composición química de la porción comestible de la Rosa aff. rubiginosa L (producto total)

Fecha muestra	Humedad g %	Proteínas (N X 5'7) g %	Cenizas g %	Extracto etéreo g %	Acido ascórbico mg %	Vit. C total mg %
2-I-73.	68'07	2'74	2'63	0'16	513	597
17-I-73.	68'53	2'53	2'48	0'40	641	730
28-I-73.	67'92	1'98	2'60	0'38	547	561
13-II-73.	67'64	1'35	2'60	0'38	714	725
3-III-73.	61'43	1'13	1'94	0'31	745	799
18-III-73.	58'49	1'06	2'10	0'31	734	737
4-IV-73.	54'25	1'06	2'11	0'36	775	787
23-IV-73.	54'15	1'30	1'98	0'26	844	849
8-V-73.	56'38	1'66	2'44	0'25	841	847
11-VI-73.	51'51	1'28	2'27	0'46	90	388

TABLA 2

Composición química de la porción comestible de la Rosa aff. rubiginosa L (referida a materia seca)

Fecha muestra	Materia seca g %	Proteínas (N X 5'7) g %	Cenizas g %	Extracto etéreo g %	Acido ascórbico mg %	Vit. C total mg %
2-I-73.	31'93	8'58	8'24	0'50	1.607	1.870
17-I-73.	31'47	8'04	7'88	1'27	2.037	2.320
28-I-73.	31'57	6'19	8'08	1'18	1.705	1.748
13-II-73.	32'34	4'17	8'04	1'17	2.208	2.236
3-III-73.	38'57	2'93	5'03	0'80	1.932	2.072
18-III-73.	41'51	2'55	5'06	0'75	1.768	1.775
4-IV-73.	45'75	2'32	4'61	0'79	1.693	1.720
23-IV-73.	45'85	2'83	4'32	0'57	1.841	1.852
8-V-73.	43'62	3'80	5'59	0'58	1.928	1.942
11-VI-73.	48'49	2'64	4'68	0'95	222	958

control del 11. VI. 73, fecha en la cual la muestra se presenta con signos de fermentación.

Lo mismo se observa en la Vitamina C total. Llama la atención que en ambos casos, las cifras obtenidas en los análisis del 28.I.73 muestran valores inferiores a la tendencia.

La Tabla N.º 2, muestra los resultados obtenidos en los análisis de la porción comestible de mosqueta, referidos a materia seca. Al realizar el estudio de los datos, se observa que:

a) la proteína disminuye notablemente, especialmente si se comparan los tres primeros resultados, con el resto. El penúltimo control muestra una leve alza en la tendencia.

b) la ceniza también presenta una tendencia a la disminución, no siendo tan

marcada como el caso anterior. También se observa que el penúltimo control muestra una leve alza en la tendencia.

c) el extracto etéreo igualmente, tiende a disminuír, con un ligero repunte en el control final (11.VI.73).

d) el ácido ascórbico muestra una tendencia a aumentar hasta el control del 13.II.73, notándose sí, una caída en el control del 28.I.73, época de transición a fruto maduro; luego, la tendencia es a bajar, hasta el 4.IV.73, para producirse a continuación un alza hasta el control del 8.V.73 y finalmente, caer en la última determinación.

Lo mismo se observa con respecto del ácido ascórbico total, no siendo tan marcada la caída final.

TABLA 3

Estudio de la composición química de la semilla de la Rosa aff. rubiginosa L (producto total)

Fecha muestra	Humedad g %	Proteínas (N × 5'7) g %	Cenizas g %	Extracto etéreo g %	Acido ascórbico mg %	Vit. C total mg %
2-I-73.	38'98	4'10	1'11	1'84	–	–
17-I-73.	25'47	4'87	1'16	4'27	–	–
28-I-73.	25'86	5'15	1'53	5'60	–	23'41
13-II-73.	22'90	4'91	1'20	4'90	–	29'12
3-III-73.	23'38	5'03	1'08	5'59	–	37'51
18-III-73.	21'60	5'34	1'23	3'56	10	37'00
4-IV-73.	19'72	5'86	1'21	5'97	–	41'00
23-IV-73.	21'80	5'36	1'26	4'24	–	48'00
8-V-73.	23'87	4'81	1'15	4'24	23	40'00
11-IV-73.	20'32	5'16	1'16	6'27	5'75	43'00

Del estudio de la Tabla N.º 3 se observa que la semilla presenta valores de:

a) humedad máxima es el mes de enero para caer gradualmente hasta el mes de abril, en donde se obtiene el valor mínimo. Luego se presenta una subida hasta el mes de mayo, para decaer nuevamente hasta el último control.

b) proteínas bastante constante, a pesar de las modificaciones de humedad.

c) cenizas y extracto etéreo, con características semejantes al caso anterior.

d) vitamina C total, bajas comparadas con los resultados obtenidos en porción comestible; razón por la cual sólo se hizo algunas determinaciones de ácido ascórbico libre.

Desde fines de enero, en que se inició la determinación, se nota un aumento en los resultados, hasta el mes de mayo, para decaer en los controles posteriores.

La Tabla N.º 4 muestra los resultados de la composición química de la semilla de mosqueta, referidos a materia seca.

En ella se observa que los valores obtenidos en proteínas y cenizas, no muestran una variación estacional. El extracto etéreo entrega valores dispares, sin que se note una tendencia.

En la vitamina C total, se ve un aumento constante hasta el control del 23.IV.73, para luego producirse una caída en los resultados.

Si se compara los resultados obtenidos en el presente trabajo, respecto del contenido en Vitamina C total, con trabajos realizados por otros investigadores, se

TABLA 4

Composición química de la semilla de la Rosa auff. rubiginosa L (referida a materia seca)

Fecha muestra	Materia seca g %	Proteínas (N X 5'7) g %	Cenizas g %	Extracto etéreo g %	Vit. C total mg %
2-I-73.	61'02	6'72	1'82	3'02	–
17-I-73.	74'53	6'53	1'56	5'73	–
28-I-73.	74'14	6'94	2'06	7'55	31'57
13-II-73.	77'10	6'37	1'56	6'36	38'00
3-III-73.	76'62	6'56	1'41	7'30	48'95
18-III-73.	78'40	6'81	1'57	4'54	47'20
4-IV-73.	80'28	7'30	1'51	7'44	51'07
23-IV-73.	78'20	6'85	1'61	5'42	61'38
8-V-73.	76'13	6'32	1'51	5'57	52'54
11-VI-73.	79'68	6'47	1'45	7'87	53'96

puede indicar que nuestros valores son inferiores. Investigadores alemanes, obtienen en porción comestible referida a materia seca, 3.112 mg % (9-X) y nosotros, 2.236 mg % (13.II) (Schwarze, W. et als, 1952). Esto se puede explicar por tratarse, seguramente, de variedades diferentes, además desarrolladas en terrenos y condiciones climáticas diferentes.

Del estudio realizado, se concluye que la *Rosa aff. rubiginosa L.*, en su porción comestible presenta contenidos bajos de proteínas, cenizas, y extracto etéreo, mostrando valores altos de ácido ascórbico libre, que difiere levemente del contenido en vitamina C total.

En cuanto a la "semilla", presenta contenidos bajos en los nutrientes fundamentales, lo que no permite pensar que sea una fuente de materia prima para la elaboración de piensos y/o para la obtención de aceites. Sin embargo, muestra la presencia de cantidades de vitamina C total tan alta como la contenida en 100 ml de jugo de limón (Beaton, G., 1964).

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Whole grain wheat, a nutritious snack food

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ABSTRACT

Snack foods supply quick energy but are often loaded with empty calories. More nutritious, yet palatable, snack foods are needed. Whole wheat is known to contain a reasonably good quality protein, essential minerals, and some of the B vitamin complex. A freeze-dried whole grain snack food was developed by steeping, autoclaving, freezing, freeze-drying, and seasoning. The amount and timing of adding salt and antioxidant, and the amount of anhydrous milk fat to be added were studied. Most acceptable were 2 % popcorn salt and 30 % anhydrous milk fat.

The product can also be used in candies, cookies, or substituted for nuts in most recipes. It can be reconstituted and used in place of rice as a carrier for meat dishes. A baked caramel coating was used to make it more palatable for snacking.

INTRODUCTION

Snack foods are popular and are gaining in popularity. In 1971, it was estimated that 96 % of the households in the United States purchased snack foods (Collins & Sanders, 1973), and the projection is for an annual growth rate of six percent in the snack food industry for the next several years. Since many of the snack foods are calorie loaded, and thus often comprise a significant portion of the daily dietary intake, it is obvious that some of these snack foods are logical candidates for supplementation with protein and other essential nutrients. Whole wheat can be the base for a snack food that is highly nutritious and very palatable.

Wheat has been the "staff of life" for a large portion of the earth's people for thousands of years. Wheat foods provide an important segment of our dietary intake at a relatively low cost. (Great Plains Wheat, Inc., 1971).

Whole grain or whole kernel wheat contains a resonably good quality protein, many essential minerals and some of the B vitamin complex. Table I was compiled from data available in the United States Department of Agriculture publication

*deceased

TABLE 1

Chemical Analysis of whole grain wheat, whole wheat flour, extracted flour & enriched flour/100 mg[a]

Variables	Food H_2O %	energy calories	Protein %	Fat gm	Carbohydrates Total gm	Carbohydrates Fiber	Ash gm	Ca mg	P mg	Fe mg	Na mg	K mg	Thiamine mg	Riboflavin mg	Niacin mg
Hard red winter.	12.5	330	12.3	1.8	71.7	2.3	1.7	46	354	3.4	3	370	0.52	0.12	4.3
Hard red spring.	13.0	330	14.0	2.2	69.1	2.3	1.7	36	383	3.1	3	370	0.57	0.12	4.3
Whole wheat flour.	12.0	333	13.3	2.0	71.0	2.3	1.7	41	372	3.3	3	370	0.55	0.12	4.3
Unenriched bread flour. .	12.0	365	11.8	1.1	74.7	0.3	0.44	16	95	0.9	2	95	0.08	0.06	1.0
Enrichment.										2.9			0.44	0.26	3.5

[a] Watts & Merrill 1963. Handbook no. 8.

Handbook No. 8 (1963). The comparison of whole grain hard wheats with whole grain flour and unenriched bread flour (80 % extraction) shows the loss of elements when the bran is removed from the wheat kernels. Shown also are areas of enrichment commonly used to bring some of the essential nutrients back to a level comparable to that of whole grain wheat.

Because of concern about the so-called "empty calorie" snack foods, a nutritious whole wheat snack food was developed and tested for nutritive value and consumer acceptance.

EXPERIMENTAL PROCEDURE AND RESULTS

At the beginning of the study wheat of unknown origin was cleaned and use for experimental purposes. Once a repeatable process was developed yielding an acceptable product, the South Dakota Seed Foundation supplied three varieties of wheat common to South Dakota: hard red winter (HRW), hard red spring (HRS) and durum. The initial procedure was as follows:

1. Steep wheat at 82° C (180° F) for 6 hrs.
2. Autoclave at 121° C (250° F) for 1 hr.
3. Freeze
4. Freeze-dry
5. Season

Sensory panel evaluations and subsequent statistical analyses were used throughout the experimental stages. To allow sufficient refinement of each variable considered, a seven-point scale was developed as shown in table 2. Occasional use was made of paired comparisons.

TABLE 2

Taste panel evaluations

Score	Appearance and flavor	Crispness	Tenderness
7	Excellent	Extremely crisp	Tender
6	Very good	Very crisp	Very tender
5	Good	Crisp	Moderately tender
4	Fair	Sl. crisp	Sl. tender
3	Sl. undesirable	Sl. hard	Sl. chewy
2	Poor	Hard	Moderately chewy
1	Very poor	Very hard	Not chewable

Preliminary study of varietal differences indicated that Durum wheat had the best appearance and was more crisp and more tender, although the HRS wheat had a much better flavor. To improve the flavor of the finished product, salt (2 % by weight) was added during the steeping process, either at the beginning of the steeping period or 30 minutes before the end of the period. Though crispness, flavor ($P < .01$), and tenderness ($P < .05$) were best when the salt was added early, the overall appearance was much better ($P < .01$) when the salt was added later. From taste panel comments, the overall appearance was more important, thus salt was added at the end of the steeping period. Eastman Tenox No. 7, a water soluble antioxidant, was added at. 0.35 % by weight of the raw grain when the salt was added.

While hard red spring wheat was again selected as the best all around product, some of the panelists expressed a desire for more salt. Salt at the levels of 1.7, 2.7 and 3.3 % was added at the end of the steeping period, with no discernible effect on appearance. However, tenderness and flavor of the product prepared with the salt level of 3.3 % were preferred ($P < .01$).

The cooked wheat was frozen at -50° C (-60° F) before freeze-drying. Huber (1971) of the National Aeronautics and Space Administration reported that freeze-dried instant rice was more tender when frozen at -23° C (-10° F) for two hours and then thawed at room temperature (22° C, 72° F) for 2 hours. Repeating this process twice greatly improved the tenderness of instant rice. Cooked wheat was subjected to freezing and thawing one, two, and three times, with a more tender product obtained from two freeze-thaw steps than from one. A third thawing had little effect on the tenderness of kernels. It is believed that this process is effective because freezing and thawing may break down some of the cellular walls and membranes to yield a more tender product (Huber, 1971). Freeze-drying was done in a Virtis USM-15 laboratory-size freeze-dryer with the following conditions: shelf temperature at -60° C, condenser at -50° C, vacuum 100-120 mm of Hg., and heat applied at 49° C (120° F). Five hundred grams of wheat was cooked at a time, spread evenly on a freeze-drying tray with 2 trays per operation, and dried until the product temperature returned to 22-25° C (17-18 hrs).

As a result of the treatments described, a crisp product was developed which then served as the basis for a snack food. Since the freeze-dried wheat was quite hygroscopic, additives of low moisture content were required. Anhydrous milk fat (a purified milk fat product) was added after freeze-drying (30 % by weight of original grain sample). Fat was melted, and the freeze-dried wheat mixed into it, then salt (2 % by weight) was sprinkled over it, mixed and heated in a 93° C (200° F) oven for 15 mins., stirring twice. A paired comparison was made, using either salt or fine popcorn salt. The fine grain popcorn salt was more acceptable ($P < .01$) in all areas of evaluation.

Another test was run with the finished seasoned freeze-dried wheat, now named "whea-ets", to evaluate the saltiness of the product. Products obtained with each of the three levels of salt added during the steeping process (1.7, 2.7 and 3.3 % by weight) were evaluated after addition of the anhydrous milk fat and popcorn salt. The lower level of salt gave a more crisp product ($P < .05$) but flavor and appearance were best with the highest amount of salt (3.3 %).

Storage:

Now that an acceptable product had been developed, its shelf life had to be determined. Six varieties were processed: HRS Chris, Durum-Leeds, hard white winter wheat and the three varieties mentioned previously, HRS, HRW and Durum. Each variety was sealed in easy-open aluminum cans (208 x 207) or in heat-sealable polyester film pouches. Samples were sealed with or without antioxidant and plain or seasoned with anhydrous milk fat and salt. Following 6 months of storage at room temperature (approximately 22°C), taste panel evaluations were made comparing the fresh product after canning or bagging to the stored products. No attempt was made to prevent minor fluctuations in room temperature during the storage period.

Analysis of canned samples and polyester pouches with fresh products and those stored for six months, indicated that freshly sealed samples scored highest ($P < .05$) for crispness, tenderness and flavor. The seasoned product (anhydrous milk fat and salt) was greatly improved ($P < .01$) over the plain product with and

without antioxidant. Effects of the storage vehicle on the keeping quality were minimal except that the canned product was more tender ($P < .05$). Comparison of varietal differences and many other interactions indicated the hard red spring wheat had the best flavor regardless of seasonings or methods of storage.

Caramel-coated whea-ets:

Several types of flavorings or coatings were considered in an effort to obtain other snack-type products based on the freeze-dried wheat (whea-ets). Among these was a caramel coating containing 212 gm. margarine, 200 gm. brown sugar, 80 gm. light corn syrup, 2.5 gm. salt, and 1.3 gm. soda. These quantities provided enough caramel to coat a 500 gm. batch of wheat. Margarine, sugar, syrup and salt were blended and boiled at medium heat to 120° C (248° F), then soda was stirred in until foamy. While making the caramel, the whea-ets were warming in a 121° C (250° F) oven. Hot caramel was poured over the warmed whea-ets, mixed thoroughly, and the mixture spread evenly in pans and returned to the oven for 45 minutes, stirring at 15 minute intervals.

DISCUSSION

South Dakota, in the Great Plains wheat belt, grows three varieties of wheat — hard red sping, hard red winter and Durum. Table 3 gives the proximate analyses of the three varieties plus analyses of the freeze-dried product, all reported on a moisture-free basis. Protein content of South Dakota wheat is above average,

TABLE 3

Proximate analyses of raw whole grain and freeze-dried wheat 3 varieties

Components[a]	Moisture %	Fat %	Protein (5.83)[c]	CHO %	Ash %	Iron (mg)	Phosphorus (mg)	Thiamin (mg)	Riboflavin (mg)	Niacin (mg)
Hard red winter										
Untreated.	10.2	1.7	18.3	80.3	1.7	12.3	322	1.09	0.13	4.88
Freeze-dried. . .	4.6	2.3	18.6	79.5	1.7	7.3	330	0.44	0.08	4.57
Retention %. . .							102	40	60	94
Hard red spring										
Untreated.	10.0	1.1	20.0	79.3	2.2	9.3	478	1.14	0.16	6.94
Freeze-dried. . .	4.3	2.0	20.8	77.7	2.2	15.1	492	0.46	0.09	6.75
Retention %. .							103	40	55	97
Durum										
Untreated.	9.8	1.9	15.6	82.4	2.2	5.6	471	1.02	0.14	9.50
Freeze-dries. . .	3.9	2.5	15.9	81.9	2.2	5.5	473	0.55	0.09	9.48
Retention %. .							100	54	64	100
Average[b] Composition of wheat. . . .	10.0	1.9	13.3	—	1.3	—	—	0.55	0.13	6.36

[a] Values reported on moisture-free basis.
[b] Pomeranz (1971).
[c] Watts and Merrill, 1963, and D. B. Jones, 1931, Whole Kernel Wheat, Conversion factor of 5.83 for N to protein.
[d] Probably contaminated with iron during grinding.

with hard wheats ranging from 15.6 to 20.0 percent protein compared to 13.3 percent average as reported by Pomeranz (1971).

The mineral element iron is found in the bran of the kernel and thus is not lost during processing steps employed in these studies. Retention of phosphorus, thiamine, riboflavin, and niacin was also very gratifying (table 3). Table 4 gives the percentages of amino acids in these three varieties of wheat (whole kernel and freeze-dried product). Again compared to the average for wheat, the high quality of the proteins in South Dakota hard wheats is apparent.

TABLE 4

Per cent of amino acids in whole kernel wheat and freeze-dried wheat (3 varieties) (Moisture free basis)

Amino acid	Hard red winter		Hard red spring		Durum		Average composition of wheat
	Untreated	Freeze-dried	Untreated	Freeze-dried	Untreated	Freeze-dried	
Lysine	0.53	0.53	0.54	0.54	0.41	0.42	0.50
Histidine	0.49	0.49	0.49	0.50	0.38	0.41	0.30
Arginine	0.98	0.96	1.04	1.10	0.79	0.81	0.80
Aspartic acid	1.10	1.07	1.06	1.06	0.81	0.78	–
Threonine	0.63	0.63	0.59	0.56	0.46	0.45	0.40
Serine	1.05	1.05	1.00	1.02	0.78	0.76	–
Glutamic acid	6.76	6.82	6.92	6.59	5.34	5.15	–
Proline	2.45	2.39	2.17	2.39	1.94	1.91	–
Glycine	0.88	0.90	0.85	0.88	0.60	0.58	–
Alanine	0.75	0.76	0.72	0.75	0.56	0.55	–
Valine	0.95	0.95	0.92	0.97	0.75	0.75	0.60
Methionine	0.32	0.33	0.30	0.34	0.22	0.20	0.20
Isoleucine	0.77	0.77	0.74	0.79	0.64	0.64	0.60
Leucine	1.45	1.48	1.43	1.51	1.22	1.23	1.00
Tyrosine	0.68	0.68	0.69	0.70	0.53	0.51	0.51
Phenylalanine	1.01	1.01	1.01	1.05	0.84	0.80	0.70

[a] Pomeranz, Y. (1971).

TABLE 5

Calorie Values for 100 grams of oiled and salted popcorn and freeze-dried wheat with anhydrous milk fat and salt

	Calories	Protein	Fat	CHO
Popcorn (100 gm)	456	9.8	21.8	59.1[a]
Whea-ets (100 gm)	636	14.6	32.0	54.4
Box of popcorn 60 gm	300			
Bag of whea-ets 20 gm	127			
Caramelized	993 cal/100 gm			

[a] Watts and Merrill 1963. Handbook no. 8.

There was some initial concern that nutrients may be lost in the steeping liquid drained from the wheat before autoclaving. Protein analyses made on the

cooking media after 6 hours of steeping time indicated a minimal amount of protein was lost (Hoffman & Johnson, 1972, unpublished data S. D. S. U.).

Whea-ets may be compared to popcorn in mode of utilization. Table 5 gives the calorie values for oiled, salted popcorn and the seasoned whea-ets in 100 gm. portions. On a single serving basis, a theater size box of popcorn weighs approximately 60 gm. and has about 300 calories, while a serving of whea-ets would probably weigh about 20 gm. (figure 1) and contain approximately 127 calories. Caramel coating the whea-ets raises the calorie content to 993 calories per 100 gm. of product, and concomitantly decreases the effective percentage of the other essential nutrients. Added flavor, however, may increase the use of the product.



Fig. 1.–Left side – 20 gm bag of seasoned whea-ets. Right side – 20 gm bag of caramelized whea-ets.

Plain freeze-dried whole wheat has a delightful nut-like flavor that blends well with other foods. Reconstitution may be effected by putting the freeze-dried wheat into cold water (1 gm/1.5 ml), bringing to a boil, covering and removing from heat, and steeping for 10 to 15 minutes. The reconstituted product can be substituted for rice in many meat dishes. The seasoned freeze-dried wheat product may also replace all or part of the nuts in cookies and confections that have little moisture added. Another excellent use is as a crumb topping for fruit desserts. Homemakers will surely discover other uses if the product is marketed.

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Reference to a company or product name does not imply approval or recommendation of the product to the exclusion of others that may be suitable.

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Studies concerning the utilization of venezuelan coconut (*cocos nucifera* L.) A. Composition. B. Storage stability of products

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ABSTRACT

The objectives of this study were: 1. Analysis of coconuts at three different stages of maturity grown in three areas of Venezuela, in relation to the quality and relative costs of production of manufactures products. 2. Determining the storage stability of two basic products, canned pulp (cocada) and desiccated coconut shreds.

The two main producing areas (600 kms. apart) did not show significant differences in chemical composition. However, the yield of solid endosperm was greater by 16.7 % in Estado Sucre than in Estado Falcon. Coconuts in Distrito Federal were inferior both in total quantity of solid endosperm and in its composition. Practical conclusions may be reached regarding source preference.

Storage of products for 150 days at 15,25 and 45°C showed the following: In desiccated shreds, slightly detectable rancidity reaching above 7.56 μg. methyl-2-undecanone and a darkening effect up to $\Delta E = 4.3$ was detected only at 45°C. In canned pulp no detectible rancidity was reached, the highest reading being 5.92 μg. Products prepared from ripe coconuts showed a slight tendency for darkening at 45°C, as compared with the less mature nuts.

INTRODUCTION

The coconut is one of the most valuable fruits of Venezuela. The production of over 40,000 tons per annum of kernel places it among the most prominent fruits of the country in line with the citrus, papaya, banana, mango and avocado. In addition, the coconut is a very important source of food and income in two distinct areas of the country, in the coastal States of Falcon and Sucre which are 600 kms. apart.

Despite the voluminous literature extolling the value and virtues of the coconut palm as the "tree of life" or the "sovereign of the trees", the products of the

coconut palm have constantly had to contend with two negative realiste the effect of which renders the generous characteristics of the palm almost out of the reach of human benefit. One is the relatively low yield per palm and per area of production in regions which are not optimally suitable for coconut production, and where little or no effort is put to correct the defficiency. In this category we may classify the lack of basic knowledge and means for combatting diseases which debilitate and destroy the palm. In the second category we have the relatively inefficient and wasteful utilization of the fruit where less than five percent of the fruit weight is used as oil and the rest is either marketed as a cheap by product or in many cases totally wasted.

In our program of the improvement of the utilization of the edible portion of the coconut, the first stage was devoted to the evaluation of the characteristics of the raw material produced in the country and the study of the storage stability of desiccated and canned coconut under local conditions.

The composition of coconut kernel as reported by Leung (1961), Craviota et al. (1951) and Cook (1951), varies considerably in their most sought after components. Thus a difference of 37 % in protein and 32 % in fat content are recorded above the lower figures. Likewise, great differences are recorded in the yield per unit area ranging from about 400 kg. to over 6000 kg. per hectare in commercial and experimental plantations (Fremond et al., 1969). The production of kernel per nut shows differences of up to 49 %.

The above figures indicate that no serious attempt to promote the utilization and propagation of coconut should be attempted without prior knowledge of the actual characteristics in the area involved.

Planification for the manufacture of storable products from the kernel necessitated information regarding the stability of the products under prevailing climatic conditions. Two of the products, desiccated shreds and canned pulp were subjected to storage temperatures which are either prevailing in areas where the products may actually be produced, or at cooler temperature which may benefit the product quality. However, the cooler temperatures may involve prohibitive investment.

Evaluation of the effects of storage time and temperature on product quality was made by measuring the development of rancidity using the method as proposed by Rasper (1965). The change of color was determined by the Hunter Color and Color Difference Method Model D-25. Both of the objective methods were compared with sensory evaluations.

MATERIALS & METHODS

Collection of samples

Coconut samples were collected from three distinct areas of the country. The two main producing areas, Estado Falcon in the west and Estado Sucre in the East. Together these two areas represent about 80 % of total national production. The third area was in the Distrito Federal which is geographically equidistant between the two main areas.

The harvesting of coconut in Venezuela is principally carried out at one of the three stages of maturity. The stage depends upon the specific use of the nut or the traditional picking method of the region. Thus the three stages of maturity which were analysed were: 1 - (7-8 months) known locally as the "cocada" maturity. At this stage the coconut "water", i. e. liquid endosperm and the gelatinous solid endosperm are directly consumed. Stage 2 - (10-11.5 months) is known as the "maduro". At this stage the nut is quite ripe and is brought down from the palm by climbing up and cutting down entire spadices. Stage 3 - (12 months or older) known as "goteado" are nuts which fall by themselves and are periodically collected from the ground. These are used only for copra production. The characteristics of

the stages of maturity were determined by a preceding investigation, Ludin (1972). Coconuts were marked at the rudimentary drupe stage and then analysed monthly until full maturity.

The figures presented in the tables are averages of ten analysis together with the calculated standard deviation.

Chemical analysis of the kernel

Dry matter-according to the method from paragraph 31.005 from A. O. A. C. (1970). Lipids - by method from paragraph 27.004 of A. O. A. C. (1970). Proteins - by method from paragraph 2.051 of A. O. A. C. (1970). Reducing sugars - according to method of Dubois et al. (1965). Carbohydrates - calculated by the difference.

Ash - method from paragraph 31.012 of A. O. A. C. (1970). Chlorides - according to the method of Hammer and Bailey as reported by Jacobs (1958).

Alkalinity of ash - according to the method from paragraph 31.017 of A. O. A. C. (1970). Results expressed as ml.H_2SO_4 1N/100 g. Titratable acidity - according to method 22.058 from A. O. A. C. (1970) and expressed as ml.NaOH 1N/100 g.

ph - by means of Potentiometer "Radiometer Model pH-M-22"

Measurement of the physical characteristics of the coconut

The following measurements were made of the coconuts. Lenght and maximal width of the entire fruit and of the nut after husking. The weights determined were of the entire fruit, the solid endosperm (kernel), the fibrous husk and the endocarp (shell). The thickness of the endocarp, the solid endosperm and the volume of the liquid endosperm (coco water) were measured.

Elaboration of products and evaluation of storage stability

Desiccated coconut was prepared by conventional methods consisting of paring of the kernel, breaking, washing, hot water treatment, shredding, drying, cooling, sieving and packaging in poly-cel bags.

Canned comminuted pulp was prepared by comminuting pared broken kernel with a Fitzpatrick Model D mill, filling cans, exhausting, sealing, sterilizing and cooling.

Both products were stored at 15,25 and 45° C. for up to 150 days and analysed every 25 days.

Two objective criteria were chosen to measure the deterioration of quality, i. e. changes in flavor due to randicity and changes in color due to darkening. Rasper's (1962) method was used to determine the development of rancidity. The method is based on the formation of a color reaction between 2,4 dinitrophenylhydrazine solution prepared in methyl alcohol and the distillate from a 50 g. sample of the coconut product containing the methyl ketones. The color develops due to the formation of hydrazones

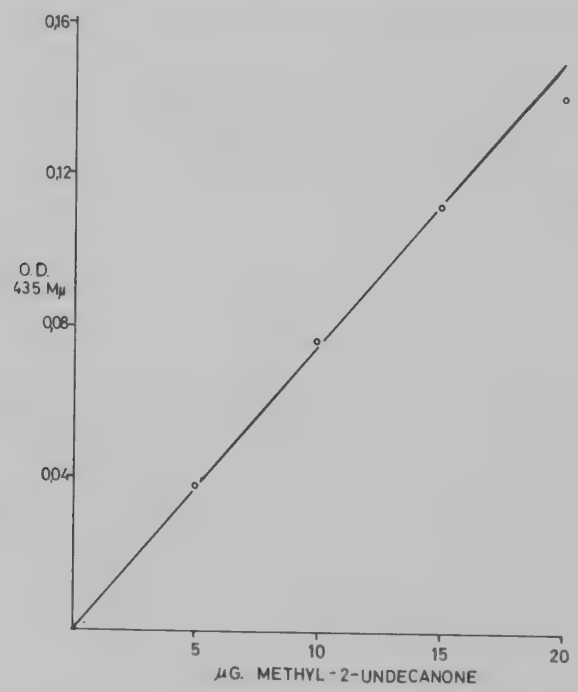


Fig. 1.–Curve for determining rancidity.

which in the presence of alkali form colored ions of quinone. The optical density was read on a Spectronic 20 Colorimeter at 435 mμ.The results are plotted on a curve of the O. D. values for 0-20 g. of methyl-2-undecanone. Fig. 1.

The darkening effect was measured by a Hunter Color and Color difference Meter Model D-25. The reference standard was a white tile having the following values L = 93.2, a = —0.8, b = 0.5. The color differences were calculated using the Hunter formula $\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$

RESULTS & DISCUSSION

A. Composition

Tables 1 and 2 present the analysis and measurements of coconuts sampled from three districts and three stages of maturity. The lipid and protein content of the most mature "goteado" nuts shows that economically this is the most advantageous stage for picking. The coconuts from the least mature "cocada" nuts, although preferred for direct consumption have less than half of the protein and the lipids, almost half of sugars and correspondingly less nutritional value. Distrito Federal lagged behind the other two areas with respect to oil content and proteins in

TABLE 1

Analysis of the components of Venezuelan coconuts from three districts and three stages of maturity

Stage of maturity	7-8 months "cocada"			10-11.5 months "maduro"			over 12 months "goteado"		
Components	Edo. Sucre	Edo. Falcon	Dto. Fed.	Edo. Sucre	Edo. Falcon	Dto. Fed.	Edo Sucre	Edo. Falcon	Dto. Fed.
Moisture, %.	73.26	73.85	73.97	43.41	43.31	44.03	36.16	36.16	38.80
	0.60	0.74	0.54	0.73	1:10	1.99	1.00	1.35	1.69
Lipids, %.	13.46	14.31	12.14	38.38	37.28	36.50	42.96	42.80	40.70
	0.74	0.88	0.81	1.33	0.81	1.21	1.41	1.21	1.68
Proteins, % N X 5.7.	2.49	2.26	2.34	3.90	3.93	3.80	4.92	5.05	4.54
	0.30	0.49	0.48	0.70	0.82	0.50	0.22	0.41	0.59
Carbohydrates, %.	9.93	8.78	10.41	13.22	12.45	14.53	15.27	14.02	15.05
	0.35	0.77	0.51	1.59	0.89	1.13	0.75	0.79	0.66
Total sugars, %.	3.58	2.32	2.34	5.81	4.26	3.85	6.28	4.72	4.23
	0.51	0.40	0.33	0.71	0.55	0.36	0.24	0.40	0.64
Ash, %.	0.72	0.79	0.78	1.10	1.04	1.23	0.70	0.73	0.82
	0.10	0.10	0.10	0.14	0.14	0.20	0.10	0.10	0.04
Chlorides, %.	0.19	0.14	0.38	0.23	0.28	0.39	0.14	0.17	0.34
	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.03	0.03
Alkalinity of ash	1.87	1.73	2.12	4.38	4.91	5.75	3.02	3.07	3.32
(ml.NaOH 1N/100 g).	0.24	0.22	0.35	0.82	0.93	0.84	0.17	0.22	0.24
Titratable acidity	1.43	0.91	1.72	2.52	2.47	2.95	2.42	2.47	3.25
(ml.H_2SO_4 1N/100 g).	0.37	0.14	0.22	0.22	0.30	0.24	0.26	0.22	0.39
pH.	6.98	6.91	6.99	6.17	6.18	6.02	6.21	6.22	5.78
	0.14	0.14	0.10	0.20	0.20	0.17	0.17	0.14	0.20

The values are averages from ten determinations with standard deviations.

TABLE 2

Physical characteristics of Venezuelan coconuts from three districts and three stages of maturity

Stage of maturity	7-8 months "cocada"			10-11.5 months "maduro"			over 12 months "goteado"		
Determination	Edo. Sucre	Edo. Falcon	Dto. Fed.	Edo. Sucre	Edo. Falcon	Dto. Fed.	Edo. Sucre	Edo. Falcon	Dto. Fed.
Length of fruit (cm).	24.52	23.16	21.63	24.61	22.51	22.28	24.39	22.57	22.67
	1.17	2.13	0.89	1.00	1.33	0.66	2.17	3.42	1.76
Width (max) of fruit (cm)...	17.62	18.55	14.99	15.31	16.69	14.39	14.11	17.07	15.24
	0.88	1.23	0.73	0.65	0.39	0.49	1.23	2.74	0.92
Length of nut (cm).	16.62	13.25	13.77	19.06	12.33	13.14	19.03	12.99	12.71
	1.07	0.60	0.49	0.66	0.55	0.62	1.79	1.33	0.87
Width (max) of nut (cm). .	10.46	11.11	10.51	12.70	8.94	10.15	12.76	10.66	9.30
	0.75	1.24	0.49	0.51	2.64	1.16	0.75	2.24	0.53
Weight of fruit (Kg).	3.978	3.608	2.733	2.257	2.038	1.765	1.177	1.033	0.930
	0.301	0.541	0.235	0.201	0.159	0.167	0.122	0.210	0.075
Weight of kernel (Kg).	0.300	0.223	0.218	0.317	0.236	0.220	0.392	0.336	0.278
	0.010	0.023	0.042	0.018	0.014	0.019	0.051	0.036	0.019
Weight of fiber (Kg).	2.864	2.582	2.006	1.625	1.495	1.292	0.470	0.419	0.414
	0.285	0.431	0.185	0.174	0.123	0.140	0.049	0.125	0.049
Weight of shell (Kg).	0.339	0.306	0.242	0.170	0.168	0.148	0.168	0.177	0.152
	0.039	0.038	0.042	0.015	0.019	0.010	0.016	0.015	0.008
Thickness of kernel (cm). .	0.86	0.76	0.81	1.18	1.18	1.16	1.27	1.27	1.19
	0.10	0.14	0.10	0.10	0.10	0.10	0.14	0.14	0.10
Thickness of shell (cm). ...	0.41	0.44	0.48	0.46	0.43	0.45	0.41	0.41	0.43
	0.09	0.10	0.03	0.07	0.08	0.06	0.05	0.07	0.03

The values are averages from ten determinations with standard deviations.

the final stage of maturity. The main significant difference between the areas of production was the weight of the solid endosperm per nut. Estado Sucre had 16.7 % more kernel per nut than Estado Falcon and 41 % more than Distrito Federal.

The characteristics of the non edible parts of the coconut as presented in Table 2 can be useful for calculating the economics of by-product utilization from the fiber and the shell.

Figure 2 shows the changes in the content of the components at the three stages of maturity. The data is the result of averaging the values from all the three districts for each stage of maturity. As indicated by the standard deviations, there is no significant difference between the different regions as to the percentage of the components in the kernel with the obvious exception of the chlorides. An explanation for the unequal uptake of the chlorides could probably be found in the quality and quantity of water available to the palm. Thus the Sucre area having the highest rainfall has the least chloride content. There is a steady increase in dry matter, protein, lipids and sugars which is evident in the relatively short interval

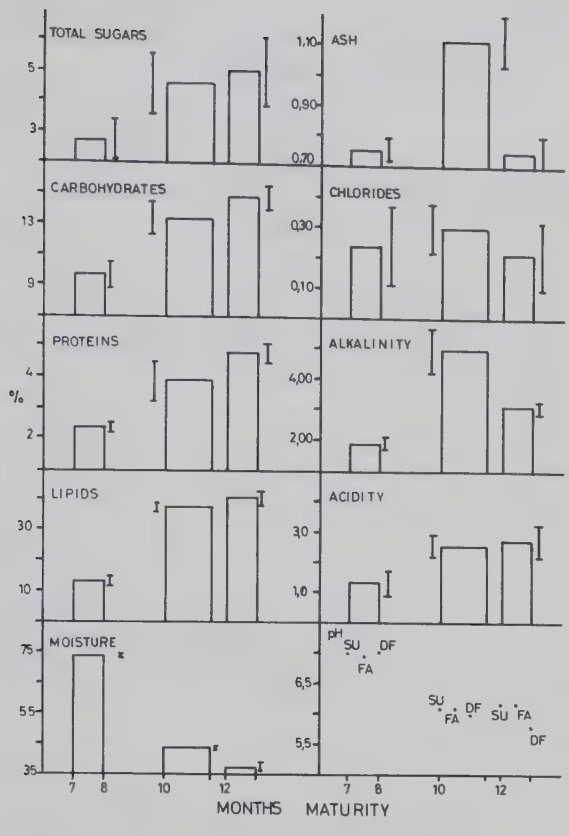


Fig. 2.–Changes in content of components in relation to maturity of the coconut (with STD. DEV.)

between the last two stages of maturity indicating the benefit of leaving the nut for longer than 12 months on the palm. The ash content showed a sharp drop after the eleventh month. Acidity increase slightly to a maximum at the second stage and remained so till the coconut dropped. The pH is reported as separate points on the graph and each one is indicated by the abbreviation of the name of the area of the origin of the samples. The highest pH was measured in Sucre and the lowest in Distrito Federal. The range for the measurements was from 5.78 to 6.99.

B. Storage stability of products

Desiccated coconut shreds and canned coconut pulp which were stored for 150 days at 15, 25 and 45° C. and evaluated every 25 days gave the following results. The development of rancidity in stored desiccated coconuts expressed in micrograms of methyl-2-undecanone is presented in Fig. 3. After 150 days at 45° C. the value reached by the product was 8.51 μg. Whereas 7.56 μg. is the limit after which a slight off odor, due to rancidity, may be detected. The samples stored at 25 and 15° C. reached only 5,3 and 3,9 μg. respectively, indicating that a much longer shelf life might be expected.

Canned coconut pulp did not show any significant difference in undecanone value and remained for 150 days, at all three temperatures, at 5.50 ± 0.35 μg. which is below the value detectable by sensory evaluation.

Color was measured at the same intervals as the rancidity. The darkening effect was expressed in Hunter L, a, b, values where a white plate (L = 93.2, a = 0.8, b = 0.8) was used as reference. With dessicated coconut, a slight darkening was detectable only after storing at 45° C for 150 days, reaching a difference of $\Delta E = 8.53$ was detected between the pulps produced from coconuts of the first two stages of maturity. The more mature raw material giving the darker color. However this difference was detected shortly after the canning operation and not due to

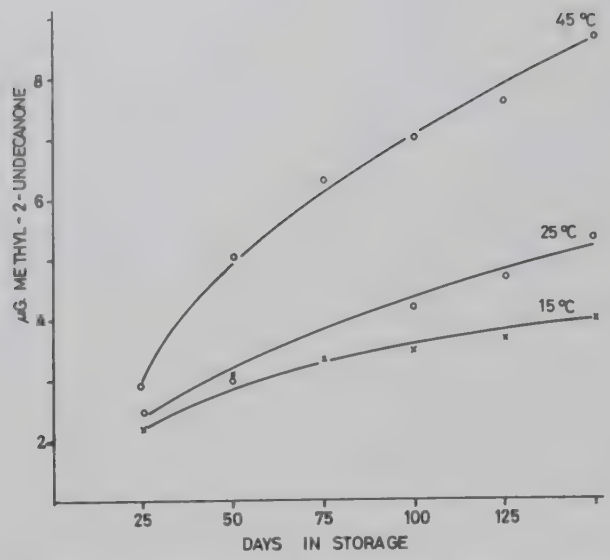


Fig. 3.–Development of rancidity in stored desiccated coconut.

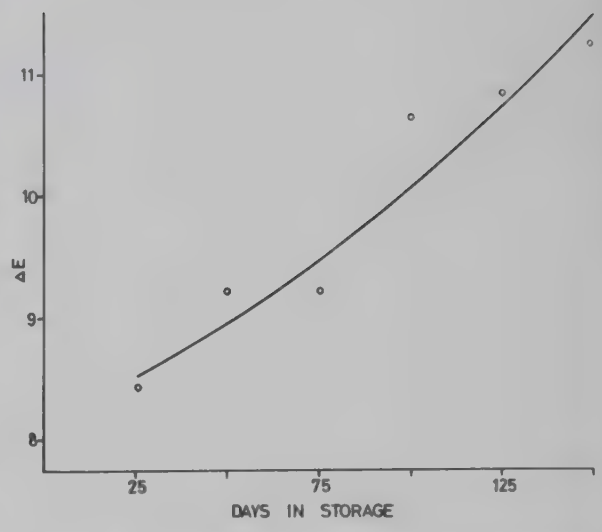


Fig. 4.–Color change (darkening) in desiccated coconut stored at 45°C.

storage. The same difference persisted throughout the whole storage period of 150 days.

CONCLUSION

The analysis of the coconuts showed that the two main producing areas do not have significant differences in their relative composition of the kernel. However, since the yield of the kernel per nut is almost 17 % higher, therefore copra or other coconut products from the kernel will be more economically advantageous in Sucre.

Analysis at the different stages of maturity show that at the final stage, when the nut is about to fall, we get considerably more and protein per nut. The only disadvantage being that it has more fiber at this stage. Though it is still possible to use the more mature nuts for the production of desiccated coconut shreds, in the production of canned pulp the less mature (7-8 month) coconut was preferred.

The problem of the fiber holds the key to the successful utilization of the coconut kernel. Consequently a concentrated study is being conducted in this direction.

Storage trials have indicated that at 150 days and relatively high storage temperature of 45° C. the initiation of deterioration can be detected. The color darkens and flavor changes due to the onset of rancidity in the case of poly-cel packaged desiccated coconut. The canned pulp did not show change due to the development of rancidity. The more ripe coconuts gave a darker canned product immediately after canning and the difference persisted at the same level throughout the storage. The practical significance of the difference in color is yet to be determined.

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High–protein breads from wheat flour fortified with peanut products

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ABSTRACT

This study was conducted to develop high-protein breads from wheat flour fortified with peanut products. Fortifying more than 10 % peanut flour or meal impaired dough property and baking quality of wheat flour. But sodium stearoyl-2 lactylate, ethoxylated monoglycerides, or sucrose monopalmitate counteracted the adverse effects, so acceptable bread was made with wheat flour fortified with up to 15 % peanut flour or 20 % peanut meal. In addition to raising the bread's protein content, its protein quality (amino acid pattern) could be improved by fortifying with peanut and soy flours. Peanut fortified bread retained softness well during storage. Its crumb appeared much darker than that of wheat bread.

INTRODUCTION

Protein-malnutrition is serious in poverty areas of many countries, where bread is an increasingly popular staple. It is an ideal food to be fortified with protein-rich additives to combat malnutrition. This study on the use of peanut products as protein-fortifiers was part of a project to develop acceptable high-protein bakery foods from wheat flour fortified with protein-rich additives by using surfactants (Tsen et al. 1971; Tsen and Tang, 1971; Tsen and Hoover, 1973; Tsen et al., 1973 and 1974; Tsen, 1974).

Peanuts are produced in many countries; China, India, Nigeria, Senegal, and the U. S. A. are major producers. Oil, of course, is the major product of peanuts. Peanut meal or flour, though largely used as protein supplements in feeds, has been reported to blend with cereals, pulses, and other products to produc new high-protein foods (Orr and Adair, 1967), but none has been commercially successful (De and Cornelius, 1971).

Available information concerning utilization research on peanut flour or meal as human food is scarce. Robinson, 1970, studied the effect of supplementary

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peanut flour on rheological properties and baking performance of American and Indian-Pakistani breads. Lorenz and Maga (1972) reported that reduced atmospheric pressures could improve the baking quality of wheat flour fortified with various protein-rich additives including peanut flour. Loaf volume and grain score of their fortified breads, however, required further improvements to make the breads acceptable.

We tried using surfactants to improve the dough property, baking quality, and shelf-life of wheat flour fortified with peanut products and compared peanut flour, meal, grits, and a blend of peanut and soy flours as fortifiers.

EXPERIMENTAL

Materials

Peanut flour I and II, grits and meal, soy flour (defatted), and wheat flour I were commercially available. Wheat flour II was milled from hard red winter wheat on a Miag Multimat in the department. Their chemical characteristics are listed in Table 1.

Calcium and soidum stearoyl-2 lactylates (CSL and SSL) and ethoxylated monoglycerides (EM), sucrose tallowate (ST), and sucrose manopalmitate (SMP) and sucrose mono- and di-stearte (SMDS) are product of the C. J. Patterson Company, Colonial Sugar Company, and Dai-Nippon Sugar Manufacturing Company, respectively. SSL was used in most of our studies because more countries have approved it as an additive for bakery products than the others.

Analyses

Proximate analyses of flours and starches were as described in A. A. C. C. Methods, 1962, except that fat was determined by the A. O. C. S. Method (Aa 4-38), 1971, using petroleum ether as the extracting solvent. Protein contents for all samples were converted by N X 6.25, except N X 5.7 for wheat flours (Table 1).

Amino acids were determined by the procedure fo Waggle et al., 1966. Bread samples, after being air dried and ground, were hydrlyzed in excess of 6N HCl for 22 hr. Essential amino acids were measured in a Beckman 120B amino acid analyzer. Methionine and cysteine were calculated as outlined by Moore, 1963, with a 24-hr. oxidation period, and hydrolyzed for 18 hr. No corrections were made for possible destruction of labile amino acids.

TABLE 1

Proximate analyses of peanut products, soy flour (defatted), and wheat flours I and II

Sample	Moisture %	Protein %	Ash %	Fat %	Carbohydrate calculated
Peanut flour I.	9.7	58.1	4.3	0.6	27.3
Peanut flour II.	8.6	56.7	4.5	1.0	29.2
Peanut grits.	8.6	58.1	4.6	0.5	28.2
Peanut meal.	8.4	51.4	4.3	9.5	26.4
Soy flour.	7.6	51.2	7.8	1.1	32.3
Wheat flour I.	13.3	12.4	0.49	–	–
Wheat flour II.	12.0	11.0	0.49	–	–

Farinograms

Farinograms were obtained by the constant-dough weight method, using 50 g. of sample (A. A. C. C. Methods, 1962).

Baking tests

The K-State (Kansas State University) Process for making high-protein bread (Tsen and Tang, 1971) was used. 70 p. p. m. potassium bromate (flour basis) was used for all the baking tests. Loaf

weight and volume averaged from duplicates were measured within 10 min. after bread was removed from the oven. Specific loaf volume (c. c. per g.) was then calculated from the average loaf weight and volume. Specific loaf volume is important for bread's marketability. Generally in the U. S. A., specific volume of marketable bread (1-lb. loaf) should be at least 6.00 c.c./g. or have a volume of 2722 c. c. with acceptable appearance, crumb texture, and grain. Bread was scored from 1 to 10, 18 hr. after baking. Finished bread scored less than 5 was regarded as unsatisfactory. Most of the baking tests were repeated at least once on a different day to substantiate results.

Unless otherwise indicated, the amount of peanut products or soy flour added to wheat flour for baking and farinographic test was expressed as percentage of wheat flour (baker's percentage).

Staling test

Staleness (firmness) was tested by following changes in crumb firmness with a recording strain gauge that registered the pressure (kg.) required to depress a one-inch-thick slice to 0.1 inch thick with a plastic plunger 1 inch in diameter. Four 1-in. slices were taken from the middle portion of each of three loaves that had been wrapped, sealed, and stored at room temperatures (about 25° C) for 1, 3, and 5 days. One pressure (kg.) reading was taken from the center of each slice. Averaged readings are reported.

Flour and crumb colors

Flour and crumb color were evaluated with Agtron multi-chromatic abridged reflectance spectrophotometer Model M-300A with monochromatic spectral lines: red (640 nm), green (546 nm), and yellow (585 nm). The instrument was standardized with standard disc M-68 to read 100 and with standard disc 00 to read 0. Flour sample was packed in a transparent cup (3.7 cm.-height, 5.5 cm.-diameter) for color measurements. For bread sample, a slice (about 1/2-in. thick), taken from th the middle portion of a loaf, was for measurements.

RESULTS AND DISCUSSION

Effects of peanut products and SSL on dough properties.

The rheological properties of wheat flour-water dough changed after being fortified with peanut products, as shown in Table 2. Dough absorption increased; stability progressively decreased as peanut flour or meal was increased. Developing

TABLE 2

Farinographic characteristics of wheat flour I fortified with indicated percentages of peanut flour I or meal

Sample	Absorption %		Developing time, min.		Stability, min	
	No SSL	0.5 % SSL	No SSL	0.5 % SSL	No SSL	0.5 % SSL
Wheat flour I + peanut flour I						
0.	65.0	64.4	8.90	13.50	13.00	22.25
12.	68.4	67.8	7.00	8.50	9.25	13.35
15.	69.3	69.3	7.05	9.60	8.15	12.70
20.	71.0	71.0	6.80	9.00	7.35	10.70
25.	72.5	72.5	6.25	8.70	6.85	10.60
Wheat flour + peanut meal						
12.	66.8	66.3	7.00	9.80	8.00	13.35
15.	67.6	67.2	6.50	9.10	7.65	10.20
20.	67.8	67.8	6.25	8.20	6.75	10.30
25.	68.5	68.5	6.00	9.00	7.90	11.10

(peak) time also decreased somewhat with fortification. The reduced stability and developing time indicate that peanut flour or meal weakens dough structure. The weakening was less with peanut meal than with peanut flour.

SSL, though it had little effect on absorption, markedly increased dough stability and development time. The dough-strengthening action of SSL seems responsible, at least in part, for improving the baking performance of wheat flour fortified with peanut products.

Baking performance of fortified flours

Preliminary tests showed that the K-State Process was better than the conventional straight-dough method such as described by Robinson, 1970, or the AACC Method 10-10, 1962, for making breads from wheat flour fortified with peanut flour. But even with the K-State Process, using more than 10 % peanut flour or meal as a fortifier drastically depressed loaf-volume and grain score of the finished bread which would be no longer considered acceptable, as shown in Table 3. On the other hand, by adding 0.5 % SSL, we got acceptable breads with specific loaf-volumes of more than 6.00 c. c./g. and grain scores of more than 5 from wheat flour fortified with up to 15 % peanut flour (Fig. 1) or 20 % peanut meal (Table 3).

Although the specific loaf volume of 6.00 c.c./g. is generally used to gauge regular white bread's marketability in the U. S. baking industry, the standard is arbitrary. Breads with specific volumes of more than 4.50 are quite acceptable (and may be preferred) in many countries. That would permit raising the fortification level of peanut products much higher.

In addition, SSL shortened proofing time for the fortified doughs. Doughs, fortified with 10 to 20 % peanut flour or meal but with no SSL, took about 90 min. proofing time to reach a template of 1.5 cm. above the center of a pan, whereas such fortified doughs reached the same height in about 70 min. when SSL was added.

Peanut meal performed better than peanut flour as a fortifier probably

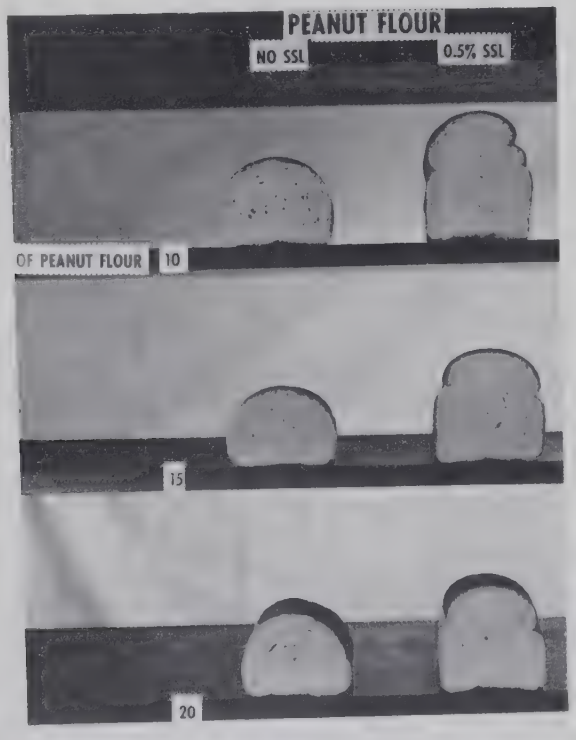


Fig. 1.–Breads from wheat flour I fortified with 10, 15, or 20 % peanut flour I.

TABLE 3

Mixing conditions, and specific loaf-volumes and grain scores of breads from wheat flour I fortified with indicated percentages of peanut flour I or meal

Fortifier %	Abs. %	Mix min.	SSL %	Sp vol cc/g	Grain score
0.	64	6.0	0	6.69	8
0.	64	6.0	0.5	7.33	9
Peanut flour					
10.	70	5.0	0	4.03	4
	70	5.5	0.5	6.60	8
15.	74	4.0	0	3.14	3
	74	4.0	0.5	6.02	7
20.	78	4.0	0	3.03	3
	78	4.0	0.5	5.05	5
Peanut meal					
12.	68	4.0	0	4.35	6
	68	4.0	0.5	6.65	8
16.	72	3.0	0	3.81	5
	72	3.0	0.5	6.30	8
20.	78	3.0	0	3.50	4
	78	3.0	0.5	6.08	7

because meal contains more fat, which improves baking quality, and it is coarser, which may affect baking performance. Tsen and Hoover, 1973, found that full-fat soy flour outperformed defatted soy flour in breads fortified with the soy flours at equal protein levels. Sieved with a Tyler Ro-Tap sifter, our peanut meal's particle size was 55.2 % over 48-mesh, 18.4 % over 100-mesh, and 26.4 % under 100-mesh, while 96.6 % of peanut flour's particles was under 100-mesh.

Effect of surfactants

We compared surfactants other than SSL for their improving effects on the loaf volumes and grain scores of breads from wheat flour I fortified with 10 % peanut flour I or meal. All surfactants tested effectively improved baking quality, particularly EM, SMP, and SSL (Fig. 2 and Table 4).

Additional experiments with wheat flour II fortified with peanut flour II and grits substantiated our results with the three most effective surfactants. In those tests, we first suspended each surfactant (EM, SMP of SSL) in 100 ml. water at 54° C and then mixed it with other ingredients into dough for beadmaking. Again, the surfactants were found effective, as shown in Table 5. Similar results were obtained with wheat flour II fortified with peanut flour II concerning the improving effect of the three surfactants.

Among the three surfactants, SMP often fave the fortified bread a slightly larger volume, while SSL provided the bread with a little better grain score. Our findings with peanut fortified breads support others' observations with soy-fortified bread (Pomeranz, 1969; Tsen et al., 1971; Finney and Shogren, 1971; Tsen and Hoover, 1973).

Peanut grits were a better fortifier than peanut flour when judged by volume

TABLE 4

Effects of surfactants on specific loaf volumes and grain scores of breads from wheat flour I fortified with 10 % peanut flour I or 16 % peanut meal

	10 % peanut flour I		16 % peanut meal	
Surfactant 0.5 % added	Sp vol cc/g	Grain score	Sp vol cc/g	Grain score
0.	4.68	4	4.35	6
EM.	6.62	8	6.21	8
SMP.	6.65	8	6.32	8
SMDS.	6.56	8	5.98	8
SSL.	6.60	9	6.30	9
ST.	6.14	8	6.03	8
CSL.	–	–	6.16	8

TABLE 5

Mixing conditions, and specific loaf volumes and grain scores of breads from wheat flour II fortified with indicated percentages of peanut grits

Fortified level, %	Abs %	Mix min.	Surfactant, 0.5 % added	Sp vol cc/g	Grain score
10	74	5	0	5.00	5
	74	5	EM	6.60	7
	74	5	SMP	6.61	7
	74	5	SSL	6.35	8
15	78	5	0	4.60	4
	78	5	EM	5.92	6
	78	5	SMP	6.00	6
	78	5	SSL	6.04	7
25	86	7	0	3.31	3
	86	7	EM	5.02	5
	86	7	SMP	5.40	6
	86	7	SSL	4.90	4

and score of breads from wheat flour II fortified with peanut grits or peanut flour II (Tables 5 and 7). The grits and flour have the same proximate composition so the variation in results seemed likely to result from differences in particle size.

Effect of particle size

We conducted additional experiments with different-sized peanut grits: particle sizes in a 56 GG sieve (fraction 1), in a 70 GG sieve (fraction 2), in a 9 XX sieve (fraction 3), and under (through) 9 XX sieve (fraction 4). The fractions contained 72.7, 12.4, and 7.1 %, respectively, of peanut grits.

Baking data (Table 6) show that fraction 1 (large particle size one) and fraction 2 (fine one) were better fortifiers than fractions 3 or 4 (both medium sized). We do not yet understand why the baking data and size of particles do not parallel.

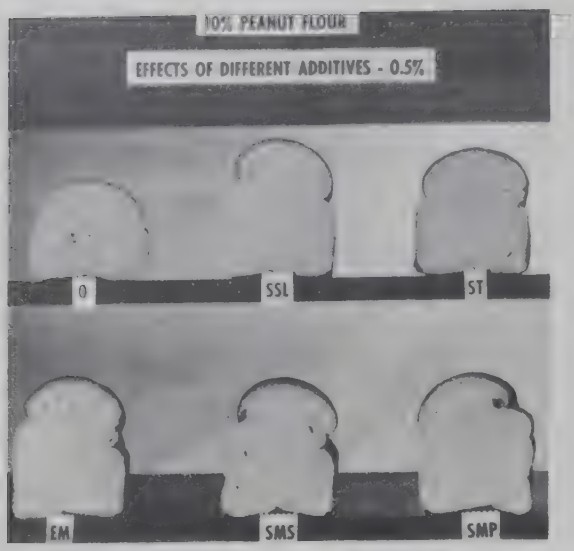


Fig. 2.–Breads from wheat flour I fortified with 10 % peanut flour I and treated with 0.5 % SSL, ST, EM, SMS, or SMP.

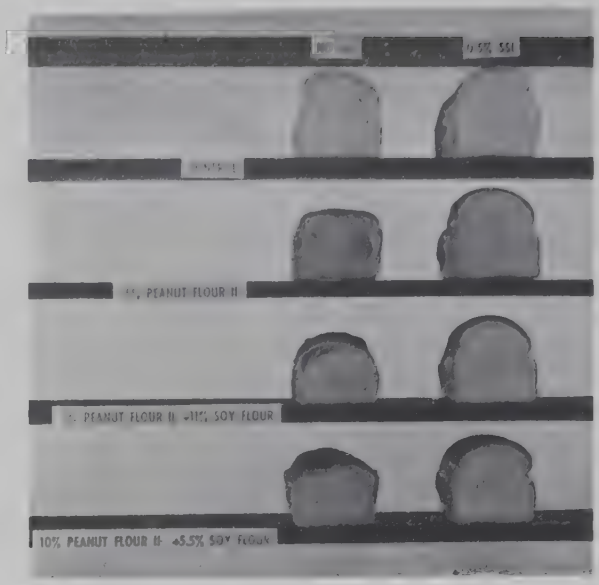


Fig. 3.–Breads from wheat flour II and indicated blends.

TABLE 6

Effects of SSL and particle size of peanut grits on specific volumes of breads from wheat flour II fortified with 15 % peanut grits

Particle size (Sieve size)	No SSL		0.5 % SSL	
	Sp vol cc/g	Grain score	Sp vol cc/g	Grain score
Original.	4.60	4	6.04	7
Fractions				
1 (over 56 GG sieve).	5.01	4	6.14	7
2 (over 70 GG sieve).	4.63	3	5.72	6
3 (over 9 XX sieve).	4.59	3	5.91	7
4 (under 9 XX sieve).	4.87	4	6.22	8

Fortified bread's protein content and quality

Table 7 shows that bread's protein contents were raised to 30.4, 43.5, and 68.8 % over the control bread by fortifying it, respectively, with 10, 15, and 25 % peanut flour II. The increase is quite impressive. Data on the mixing conditions and effects of SSL are included in Table 6 for comparisons.

Because peanut protein is deficient in methionine, lysine, isoleucine (Evans et al., 1967), and threonine (McOsker, 1962; Anderson and Warnick, 1965), we fortified wheat flour with a blend of peanut and soy flours to improve the bread's protein quality. Mixing conditions and baking qualities of such fortified breads are given in Table 7. Their protein contents were comparable with that of bread fortified with 15 % peanut flour.

To understand the benefit of such fortification, the amino acid contents of peanut flour II and soy flour were analyzed (Table 8). Chemical scores were calculated according to a provisional amino acid scoring pattern proposed by a recent joint FAO/WHO Expert Committee, 1973. Soy protein contains more lysine,

threonine, leucine, isolucine, and valine than peanut protein does, so adding soy flour should improve the protein quality (amino acid pattern) of peanut fortified brad. With the adequate supply of sulfur-containing amino acids from wheat flours, the nutritional value of the fortified breads, particularly peanut-soy fortified ones, would exceed that of wheat bread. However, the fortified bread, as predicted from Table 8, would still be deficient in lysine and threonine. Chemical scores that are based on total amino acids, not total available amino acis, tend to exaggerate the biological value of such amino acids as lysine. We are, therefore, now feeding rats to evaluate the nutritional value of fortified breads.

TABLE 7

Mixing conditions, loaf volumes, grain scores, and protein contents of breads from wheat flour II fortified with indicated percentages of peanut flour II, soy flour, and 0.5 % SSL

	Dough		Bread	
Fortifier(s) % wheat flour	Abs %	Mix min	Sp vol (score) cc/g	Protein (+ % increase) % (dry basis)
0.	60	7	6.60(9)	13.8
Peanut flour II				
10.	75	5	6.26(8)	18.0 (+ 30.4)
15.	79	5	5.72(7)	19.8 (+ 43.5)
25.	87	7	3.88(3)	23.3 (+ 68.8)
peanut flour II + Soy flour				
5.0 + 11.	75	5	5.23(7)	19.9 (+ 44.2)
10 + 5.5.	75	5	6.16(7)	20.2 (+ 46.4)

TABLE 8

Amino acid contents (mg/g protein) and chemical scores of peanut flour II, soy flour, and wheat flour

Amino acid	WHO requirement pattern content mg/g	Peanut flour II		Soy flour		Wheat flour [a]	
		Content mg/g	Chemical score	Content mg/g	Chemical score	Content mg/g	Chemical score
Lysine.	55	37	67	60	109	21	38
Threonine.	40	25	63	35	88	25	63
Methionine and cysteine.	35	8 21	83	10 10	57	14 36	143
Leucine.	70	64	91	74	106	71	101
Isoleucine.	40	32	80	39	98	33	83
Valine.	50	33	66	41	82	42	84
Phenylalanine and tyrosine.	60	50 41	152	48 34	137	48 29	128
Tryptophan.	10	–	–	–		–	

[a] Robinson, 1970; The wheat flour contained 12.5 % protein (dry basis).

Storage test

Several kinds of bread, described in Table 7, were prepared for storage tests. Peanut fortified bread retained softness well. With SSL, peanut fortified breads were much softer than wheat breads during storage. When breads were fortified with a blend of soy flour and peanut flour, however, they firmed rapidly, particularly the one with 5.0 % peanut flour II and 11 % soy flour. ST and EM gave similar results (Table 9).

TABLE 9

Average compressimeter readings (kg) stored breads made from wheat flour II and indicated blends

	Days stored					
	No SSL			0.5 % SSL		
Fortified bread	1	3	5	1	3	5
Control (wheat flour II).	6.12	6.79	7.46	5.53	6.14	6.55
15 % peanut flour II.	6.33	7.55	7.87	3.05	3.23	3.46
5.0 % peanut flour II + + 11 % soy flour.....	9.53	9.76	12.13	7.34	8.39	9.38
10 % peanut flour II + + 5.5 % soy flour.....	8.20	9.33	9.84	6.69	6.90	7.20

Crumb colors of fortified breads

Flour and crumb colors, measured by Agtron multi-chromatic abridged reflectance spectrometer, are listed in Table 10. Typical loaves are shown in Fig. 3.

The crumb of peanut fortified breads appeared much darker than that of wheat bread (Table 10 and Fig. 3). Two fators were mainly responsible for the dark-coloration: First, peanut flour was darker than wheat or soy flour. Second, more extensive brwoning reaction likely took place during baking for peanut fortified bread than for wheat bread.

TABLE 10

Agtron readings (red and yellow) of wheat flour II, peanut flour II, soy flour, and crumbs of indicated breads

Specification	Wheat flour	Peanut flour	Soy flour
Red.	54	42	50
Yellow.	45	32	40

	Bread (crumb)		Bread (crumb)					
	Wheat Flour		15 % peanut flour		5.0 % peanut flour + 11 % soy flour		10 % peanut flour + 5.5 % soy flour	
	No SSL	0.5 % SSL	No SSL	0.5 % SSL	No SSL	0.5 % SSL	No SSL	0.5 % SSL
Red..	98	100	59	83	65	88	62	80
Yellow.	87	97	45	70	49	74	46	65

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Rapeseed protein concentrate

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ABSTRACT

A new process has been developed for the production from rapeseed of a non-toxic, bland, light-coloured protein concentrate for human consumption, together with high quality oil. The rapeseed protein concentrate (RPC) has a protein content of 65 % in dry matter. Rapeseed protein has an amino acid composition better than any other vegetable protein known, and it compares well with many animal proteins. Unlike soya protein, the important amino acids methionine and cystine are present in rapeseed protein in adequate quantities.

RPC has been compared mainly to soya protein products in its functional properties, and the most striking results are its high water-bindning capacity and its freedom from off-flavour when used in foods.

INTRODUCTION

The Swedish companies AB Karlshamns Oljefabriker, Karlshamn, and Alfa-Laval AB, Tumba, have together developed a new process for the production from rapeseed of a non-toxic, bland, light-coloured protein concentrate for human consumption, together with a high quality oil. The rapeseed protein concentrate has a protein content (N X 6.25) of about 65 % on a dry weight basis. The rapeseed protein concentrate (RPC) is also usable for special feed purposes.

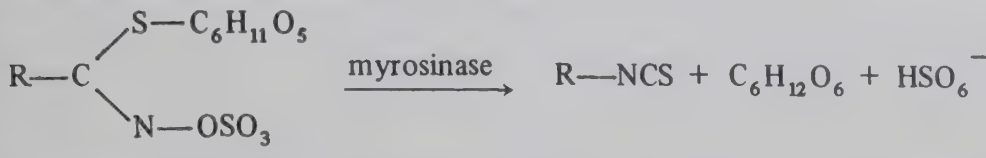
Rapeseed is one of the most important sources from which oil is produced. It is one of the five most widely produced oilseeds in the world; in addition, it is the oil-yielding plant which has gained most in popularity in the 1960's. The largest areas under cultivation are in Canada, India, Pakistan, France, Poland, Sweden, West Germany and East Germany. Rapeseed is the only oil-yielding plant which can be cultivated successfully on all continents.

In the past, rapeseed was grown solely in order to extract oil. The residue remaining after the pressing procedure can be used as fodder in limited quantities, mainly for cattle.

The main objection against the use of rapeseed meal for animal feed has been its content of deleterious glucosinolates. Through the new process, the content of glucosinolates in the concentrate is reduced to less than 1.5 mg/g, corresponding to

a content of less than 0.5 mg/g of oxazolidinethiones plus isothiocyanates, which are split products from glucosinolates.

The glucosinolates, which are constituents of the species of *Brassica* and *Crambe*, have the following general formula:



By means of endogenic enzymes, myrosinases, the glucosinolates are split into isothiocyanates or oxazolidinethiones, glucose, and bisulphate. The isothiocyanates and the oxazolidinethiones are deleterious in higher concentrations and disturb *e. g.* the functioning of the thyroid gland. This is to-day the reason for the limited use of rapeseed meal as feed.

EXPERIMENTAL

Raw material

Raw material for the process can be seeds from different species of the Brassica genus, such as rape (*B. napus*), turnip rape (*B. campestris*), oriental or brown mustard (*B. juncea*), black mustard (*B. nigra*), and white mustard (*B. hirta* or *Sinapis alba*). Crambe (*Crambe abyssinica*) can also be used in the process.

Process description

The process for the production of rapeseed protein concentrate comprises four main steps:

1. Dehulling
2. Myrosinase inactivation by heat treatment
3. Glucosinolate removal by water leaching
4. Oil extraction

The carefully cleaned seeds are crushed in roller mills, and the material is then divided into three fractions by sieving. The middle fraction from the sieves is mixed and separated into hulls and a clean meat fraction on gravity tables.

The meat fraction is then heat treated in order to inactivate the glucosinolate-splitting enzymes (myrosinases) and to lower the solubility of the protein. This is done in a screw conveyor with hot water.

The process then continues with the countercurrent water leaching of the glucosinolates in several steps.

The detoxified seed meat is dried in a fluidized bed drier under mild conditions.

The oil in the dried material is recovered by direct solvent extraction or by pre-pressing in an expeller press followed by solvent extraction.

The seed meat material is flaked by roller mills, and the temperature is adjusted in the heating pan. In the press most of the oil is taken out. The press cake is cracked in a mill, and solvent extracted in a rotary solvent extractor with a food grade hexane solvent. The remaining rapeseed protein concentrate (RPC) is freed from solvent traces under mild conditions, preferably in a "flash desolventizer" or "vapour desolventizer", so that destruction or discolouring of the protein is avoided. The oil content of the extracted RPC is 0.5 - 1.5 %.

The rapeseed protein concentrate is milled to a flour of suitable particle size. RPC can also be texturized by an extrusion process to give it a suitable coarse structure for use as a meat extender.

The leaching water from the process is concentrated in an evaporator, and the residue can either be dried together with the hulls and the pressed fines, and than used as a cattle feed, or used separately. There will thus be no pollution problem with the process water or with the process itself.

The material balance and yield for the process depend on the raw material. As an example, the following values have been obtained with rapeseed (**B. napus**, winter type):

Fraction	Yield, %
Hull fraction	16
Fines (pressed)	4
Dry matter from evaporated leaching water	18
Oil	37
Rapeseed protein concentrate	25

QUALITY CHARACTERISTICS OF PRODUCTS

Rapeseed oil

The quality of the rapeseed oil is in some respects superior to that of normal crude rapeseed oil. It has a lighter colour, the contents of phosphorus, sulphur and free fatty acids are lower than normal, and the peroxide value is in the normal range, giving an oil easy to refine and hydrogenate.

Rapeseed protein concentrate

The RPC has a composition well suited for a product for human consumption, with a protein content of approximately 65 % and a moisture content of 8 %. In *Table 1* the composition is given in more detail.

TABLE 1

Typical analysis of RPC (from B. napus, *winter type)*

Component	Content in dry matter (%)
Protein (N X 6.25)	65
Protein (N X 5.5)	57
Fat	1
Carbohydrates (exclu. fibers)	28
Crude fibers	7
Total ash	7
Glucosinolates	0.06

TABLE 2

Amino acid composition (g/16 g N)

Amino acid	RPC (from *B. napus*, winter type)	Defatted soya bean flour 1)
Isoleucine	4.4	4.4-4.9
Leucine	7.9	7.2-4.8
Lysine	6.7	5.4-6.7
Phenylalanine	3.8	4.6-5.4
Tyrosine	3.2	3.4-5.4
Cystine	2.0	0.8-1.6
Methionine	2.2	1.2-1.5
Threonine	4.7	3.6-4.2
Valine	5.6	4.0-5.2
Tryptophan	1.6	0.9-1.4

[1] **Smith, Circle: Soybeans: Chemistry and Technology, Westport, 1972.**

The rapeseed protein has an amino acid composition far better than that of soya beans, and one which compares well with many animal proteins. Unlike soya protein, the important amino acids methionine and cystine are present in rapeseed protein in adequate quantities.

The composition of essential amino acids in RPC is given in *Table 2.*

As can be seen from *Table 2*, RPC has a very good amino acid composition, which is also evident from results of rat feeding studies where the following data were obtained:

PER	2.8 – 3.8	(2.5–3.0 when adjusted to casein with a PER value of 2.5)
NPU	78	
Biological value (BV)	92	
Digestibility (D)	86	

By-products

The combined fractions of hulls, fines and dry material from the leaching water have the following composition, and could be used as cattle feed.

TABLE 3

Typical analysis of feed fraction

Component	Content in dry material %
Protein (N X 6.25).	24
Fat.	15
Carbohydrates (excl. fibers).	36
Crude fibers.	16
Glucosinolates.	9

Functional properties and field of application of RPC

In order to achieve a high acceptability of a new protein concentrate as an ingredient in foods, it should have good functional properties. This fact has often been overlooked. Rapessed protein concentrate has been compared to soya protein products in its functional properties, and the most striking results are its high water-binding capacity and its freedom from off-flavour when used in foods.

The water-absorption capacity of RPC is 500-700 %, as compared to 400 % for texturized soya bean flour, and the water-holding capacity is 400 % as compared to 200-300 % for texturized soya bean flour. RPC can also bind fat, which is useful for regulating the fat content in foods.

As compared to soya bean proteins, RPC could be used in 2-3 times higher concentrations in different foods with no detectable off-flavour.

The solubility of the protein in RPC is low, and comparable to that of heat-treated soya bean products. The nitrogen solubility index (NSI) of RPC is about 5 in pure water and about 21 in 0,2 M NaCl .

The emulsifying and foaming properties are the same as for heat-treated soya bean products.

The rapeseed protein concentrate is intended to be used as an addition to the ordinary diet. It could be used in a great many foods, such as minced meat products, sausages, canned meat products, bread, biscuits, etc.

Toxicological testing

Preliminary studies for 3 months on rats have shown that the material is safe, and no toxic effects from the small amounts of residual glucosinolates could be detected. Extensive studies of RPC with two animals and for periods up to 2 years are in progress or are planned to ensure that the products is proved to be absolutely reliable. After this, the test results will be presented to national and international authorities for final approval.

GENERAL REFERENCE FOR RAPESEED

Appelquist, L. A. and Ohlson, R., eds., *Rapeseed,* Elsevier Publ. Comp. (Amsterdam) 1972, 391 pages.

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Weaning foods in developing countries

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ABSTRACT

If local foods are to be exploited and processed it must be clearly stated what is meant by high nutritional value, to whom this must apply and for what particular purpose these foods should be used.

During the weaning period it is the needs of infants and young children which must be considered, primarily from the point of view that their requirements for energy must be met by mixtures of foods supplying enough protein of adequate quality to satisfy their fast rate of growth. Because it is not always possible to breast feed an infant, substitutes must be found, in addition to foods for the weaning period. With reasonable economic and hygienic standards the infant's requirements can be met in this way with no interference in the normal pattern of growth and development. In developing countries it is a small percentage of families that have an economic situation to permit the purchase of sufficient of these foods, usually imported, and its correct preparation in a safe and clean way.

For the much greater percentage of the population living in both rural and urban areas, the cost of feeding an infant on commercial formulas is seldom economically feasible. If practised, the nutritional needs of the child will not be met because insufficient can be bought either as a breast milk substitute or supplement. Commercially processed foods are expensive because of the necessary distribution and costs are increased further through the frequent difficulties of transport.

Apart from their comparatively high cost and, may be, their seasonal availability, other problems exist in many rural and peri-urban homes with commercial products. Where literacy rates are low and facilities for nutrition education sparse, the maker's instructions can be misinterpreted. Environmental conditions, along with shortage of equipment, safe water supplies or fuel very often lead to contamination of the infant's food.

Local low-cost mixtures have also been produced and marketed. These are usually based on plant foods grown locally and mixed to give the maximum protein value. They may have additions of minerals, vitamins and some other concentrated source of protein such as dried skimmed milk.

Unless food is eaten, it has no nutritional value. For consumption food must be palatable, culturally acceptable and available throughout the seasons in one form or another, at an economic price. Except in early life, when breast milk is the only food ingested, man's diet consists of mixtures of foods taken as meals at varying frequencies over the day.

Whether their nutritional value is high or low depends both on the amount of the foods eaten and its ability to meet needs for energy and its quality which should provide a sufficiency of all other nutrients to suit the consumer's age, sex and physical activities as well as suit his tastes for flavours and preferences for food.

During the weaning period, the nutritional needs of infants and young children are high and total energy requirements must be met by mixtures of food supplying enough protein of adequate quality along with all other nutrients to satisfy the fast rate of growth. These mixtures must also be of a consistency and bulk to suit the capacity of the stomach and although tastes for food flavours and textures are only developing at this time, palatability is another factor to be considered for the young child, his mother and possibly even this grandmother.

Breast feeding, though ideal and to be recommended, is not always possible and substitues for human milk must be found in addition to foods for the weaning or transitional period until adult foods can be taken with ease. Given reasonable economic conditions and satisfactory standards of hygiene along with an ability to follow manufacturer's instructions, infants' requirements can be met in this way without interference in the normal pattern of growth and development.

However, in developing countries at present, it is a small percentage of families that has an economic situation permitting the purchase of enough of these substitute foods, usually imported, and their correct preparation in a safe and clean way.

An important consideration for people living in both rural and urban areas in many parts of the tropical and subtropical world, is the cost of feeding infants on commercial products. Different countries have different problems. In some, the population is predominantly concerned with subsistance farming and has little access to ready cash. (For example in Malawi, about 85 % depend for their livelihood on smallholder farming). In others, (e. g. in the Caribbean islands) because crops are grown for export foods to eat must be imported and their cost is high in relation to average income.

Given these conditions, the satisfactory feeding of an infant with commercial proprietary products is seldom feasible within the family budget. Too often, if it is practised, the results are disastrous for the product cannot be bought in sufficient quantity to satisfy the needs of the infant either as a substitude for breast milk, or as a supplement. What can be afforded must last for a definite period of time, rather than provide adequate nutrition at regular intervals.

Cost of distribution add to the price of the commercial products and these may be increased further by local difficulties of road, rail or other forms of transport, which in addition quite possibly create and erratic availability of supplies.

Other problems also exist in many rural and peri-urban homes for proprietary products. Where literacy rates are low and facilities for nutrition education are sparsely scattered, the maker's instructions can be misinterpreted, even if they can be read. Of the totàl population over fifteen years of age in some countries, the literacy rate may be less than 25 % and for women it could be consideraly less than for men. Clinics or health, agriculture, home economics or community development centres may be few in number, be difficult of access for regular attendance and be

staffed by personnel with only rudimentary training and little time to spare for teaching even the most basic facts about the use of these foods for the youngest family members.

Environmental conditions, along with shortage of household equipment, safe water supplies or fuel and restrictions on the free time of the mother from other duties, very often leads to contamination of the prepared food. This reduces its nutritional value through the consequent gastro-intestinal and parasitic infections of the infant.

Local low cost mixtures have also been produced and marketed, sometimes as complete foods and sometimes as a supplement to be used with others, such as the traditional pap or porridge. These mixtures are usually based on plant foods, grown locally and in proportions which give maximum protein value. They may have additions of minerals, vitamins and some other concentrated source of protein, such as dried skimmed milk, but their overall value again depends on enough being fed at a time.

Though fairly inexpensive to produce, the mixtures must, of necessity be distributed so that regular supplies are available in appropriate districts or regional areas at an economic price. With a limited budget for food or other subsidies in a country, the price of such a mixture may still be too high for the low income groups. Many families in peri-urban areas or those depending on substance farming have little, if any money to spare for their purchase.

As a short term curative measure, these processed mixtures of food have advantages if used under the supervision of medical personnel or their assistants. They serve a very useful purpose for a child already undernourished, if a mother is shown low they should be used, how often and in what quantity. Education for this requires both trained staff, from the doctor through others to the village level, and the existence of hospitals, rehabilitation units, clinics or health centres. Depending on national policies, some of these foods may be available through the local clinics, either free or at a token cost.

On a long term basis, in these same communities there should be preventive, as well as curative measures if the unacceptably high infant mortality rates are to be reduced. Mothers and adolescent girls, even school children should be taught how to exploit the foods that are normally available from their gardens or markets, to the fullest advantage. The combination of foods recommended to be used and the processing methods for their preparation should aim at maximum retention of nutrient value, in suitable consistency and acceptable bulk to meet the infant or young child's requirementes for energy and growth.

Education for exploitation of the local foods should involve all the family, male and female alike, to ensure sufficient variety of production, adequate facilities for storage with minimum waste, along with practical advice to budget food supplies so that they last through the agricultural cycle.

Unless there is a disaster such as a drought or a flood, supplies of local foods should be readily available through out the year and even if not grown in the household garden they are likely to be of low cost in the nearby market. Of even greater advantage is that the mother is familiar with these foods; she knows their flavour and the methods for preparing them using the utensils she has at home. It is encouragement which is often the vital factor needed to persuade her to feed enough of them in a good nutritional mixture to the youngest members of he family.

In summary, for developing countries commercially processed foods for infants and young children can be used successfully by the small strata of

population having acceptable standards of living and education. At present, however, for the larger majority who cannot rely on purchasing enough foods, especially if processed or imported, to feed their families adequately, encouragement and education must be available to help them exploit the best of household methods for processing their local foods to produce meals in sufficient quantity and of maximum nutritional value.

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The decortication of some tropical nuts

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ABSTRACT

Experimental work was carried out on three entirely different types of nuts:

1. The cashew nut, with a tough leathery shell which contained anacardic acid, and a delicate kernel with a testa or skin which was difficult to remove.
2. The macadamia or Queensland nut, which was almost spherical in shape and which contained a close fitting kernel within a very hard shell between 2-4 mm thickness.
3. Ground nuts from various countries, the shells of the dry nuts were friable, and easily removed, and the testa very easily damaged. The kernels themselves split readily at the cotyledon.

The experimental work on the cashew nuts was directed toward an impact method of decortication because the aim was to develop the simplest plant for village or cooperative operation, and a centrifugal decorticator was devised which worked effectively. Various methods of extracting the kernel from the very hard shell of the macadamia led to the invention of a means of cutting the shell around its circumference and splitting it open by wedges. The work carried out on ground nuts was devoted to the improvement of the standard semi-rotary ground nut decorticator, and led to the provision of a feeding device which provided sufficient nuts to cover the screen over which the shoe moved and abraded the shell, thus removing the kernels. The method was further improved by ensuring that the correct sized screens were used in the correct order.

Cashew nuts in particular required a great deal of investigating work before correct preprocessing conditions could be obtained and the methods evolved yielded 50 to 55 % of whole nuts in the cans when packed.

Macadamia nuts required particular attention to be paid to drying rates, and the cutting method then yielded 90 % whole kernels if they were not already split at the cotyledon.

The groundnut feeder linked into the hand operating mechanism decreased the work effort required by almost a factor of two and the method of ranging the screens decreased kernel breakage and testa damage.

The conclusions reached were that correct preconditioning of the nut was essential if an efficient decortication method was required.

INTRODUCTION

The demand for confectionery nuts is increasing in the more sophisticated countries. and creates a valuable export market for many of the lesser developed countries. As a general rule the purchasers of nuts of this type insist that they are whole and undamaged and therefore whole kernels fetch a high premium. Investigations at the Tropical Products Institute have been directed toward the development of small decortication plants aimed at producing a high proportion of undamaged kernels.

Traditional methods of decorticating cashew, macadamia and ground nuts were studied as a preliminary to the development programme, and it was noted that there was considerable room for improvement. The work carried out by the Tropical Products Institute will now be discussed under the appropriate headings.

EXPERIMENTAL

1. Cashew nut processing

Since an impact method of kernel removal was required it became obvious at a very early stage that because of the peculiar characteristics of the nut considerable research and experimental work would have to be done to obtain the correct shell and kernel conditions which would allow the shell to break fairly readily and leave the kernel unharmed. It was also apparent that the impact force associated with the conditions was of vital significance.

At an early stage it was agreed that a throughput of 300 kgs of raw nuts per hour would be the type of machine required by villages or cooperatives. The capacity was kept deliberately small because very large commercial plants using a different decortication method were already in existance but had the effect of moving people from the rural areas and into the larger cities. It was considered that a centrifugal cracker should be developed. It would consist of a radially vaned, horizontally rotating disc, into the centre of which the nuts would be dropped, and thrown against target plates arranged around the periphery of the disc.

Having considered the means of cracking the nut it was then essential to determine methods of obtaining the correct 'brittleness" of the shell, at the same time retaining a resilient kernel which would withstand the impact forces. It was established that with a brittle shell, and an average nut weighing 6 grams an impact velocity of 19.6 metres per second was required. A considerable time was then spent in obtaining experimentally the correct roasting time and temperature conditions and the moisture content of shell and nut prior to roasting which would give the optimum cracking efficiency.

It was found that with a moisture content between 10-12 % in the shell and 8-10 % in the kernel a roasting temperature from 180-185° C and roasting time of 1.4 to 1.8 minutes an average of 75 % whole kernels could be obtained using Kenyan cashew nuts.

A considerable amount of effort was devoted to the design of a cashew shell fired roaster which used the gaseous products of combustion passing through a heat exchanger in the roasting tank as the means of heating cashew nut shell liquid to the required temperatures. The soaked and humidified nuts were passed by conveyor over the surface of the CNSL, through the heated liquid. During roasting 18 litres of CNSL were expelled from 300 Kg of nuts per hour and this was drawn off the tank as a by product. The roasted nuts were cleaned and cooled by passing through a rotating trommel in contact with sawdust for 7 minutes and were then fed into the centrifugal decorticator.

Experimental work had indicated that any one nut required an average of about 7 impacts against the target plates before the kernels were released. It was therefore necessary to arrange a recycling system, and the mixture of shell and kernel was separated by means of air eluctriation. The kernels left the decorticator at 7 to 8 % mc, and it was then necessary to dry them to less than 5 %. This low moisture content made in easier to remove the testa and was also essential for storage when canned. The dried operated at 85-90° C and was fuelled by cashew shell. After drying the kernels were passed through a continuous cooler held at -5° C to -6° C, and this caused the testa to contract and split. The kernels then passed through a rotary peeler and approximately 70 % of the kernels were completely cleaned, the

remaining 30% had small pieces of testa adhering to them which were readily removed by hand during the grading and inspection operation. About 55 % of whole kernels were canned using this relatively simple plant.

2. Macadamia Nut Decortication

These table nuts are in considerable demand in America, and are mainly produced in Hawaii. Several developing countries are interested in entering the market for this very lucrative export item, because the macadamia is probably the most expensive confection nut in the world. The Tropical Products Institute decided to develop plant and equipment to decorticate this nut and considered several methods such as the use of rollers, and vices. It was found however that the shell was so hard, and the kernel in such close contact that depression of the shell frequently resulted in a damaged kernel. Il was obvious that the method of drying before decortication was of great importance, because it was essential that the almost spherical shell should remain uncracked, but at the same time the kernel was required to shrink sufficiently to allow it to be removed readily. The considerable amount of literature available showed that drying times from two to six weeks in good storage conditions and with air circulation was necessary.

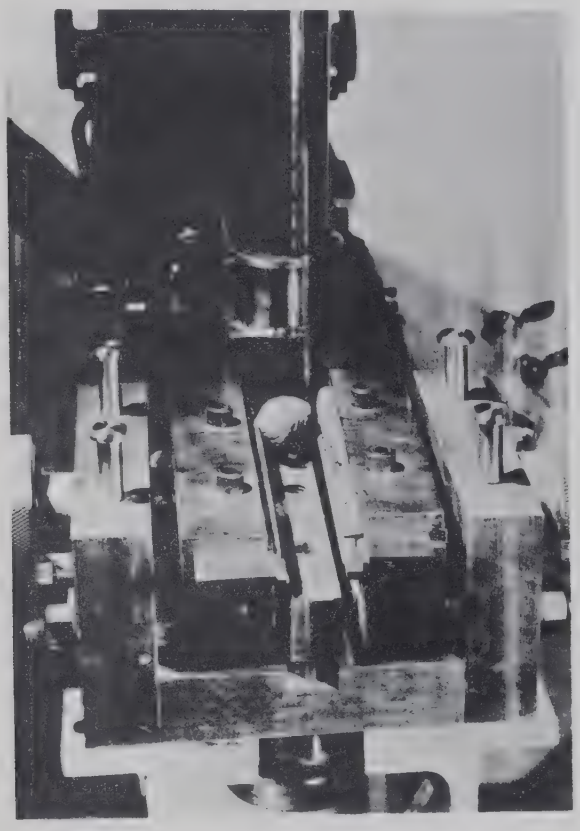


Fig.–Macadamia nut decorticating unit.

After considerable experimental work a new means of removing the kernel was devised. This consisted of a horizontal air operated ram which pushed the spherical nut between two saw toothed guides and a knife edge. The movement forward caused the nut to rotate when in contact with the teeth, and the knife was set to cut a groove around the circumference of the nut without touching the kernel. At the end of the stroke small wedges engaged in the groove caused by the knife and forced the two halves of the shell apart, thus ejecting the kernel. This machine produced about 90 % of whole kernes but it was clear that the method of drying had a considerable effect on the product. Cracks in the nut resulted in pieces of the shell cutting into the kernel during decortication. It was also noticed that the cotyledon had already separated before the decortication took place if the nuts had been in store too long. From experimental trials it would appear that although this machine, one unit of which handles 45 kg of nuts per hour, has a lower throughput than other available machines, the quantity of whole kernels produced makes it a viable proposition.

3. Groundnut decortication

During recent years considerable interest has been shown in the improvement of methods of decorticating ground nuts, particularly in villages. For many years the standard semi-rotary decorticator has been in use. This consists of a container with a curved mesh screen as its base, with a hand-operated serrated shoe which moves over the surface of the screen, the clearance between screen and shoe being greater than the diameter fo the kernel and smaller than that of the nut. Work carried out on these machines in Madagascar showed that they were not being operated correctly and that improvements could be made to the machine. It was noted that the method of operation was to fill the container with nuts before decortication started. The nuts within the container were therefore impeding the movement of the lever and shoe. Measurements showed that almost twice as much work was being done by the operative than was actually required since it was only necessary for sufficient nuts to cover the screen to be in the container during any one movement of the handle. A feed device linked to the operating lever was fitted, and this discharged the correct quantity of nuts with each arm movement and resulted in decreasing the effort required by a factor of two and also slightly decreased damage to the kernels. Further examination showed that it was much more efficient to use three machines in series, the first being fitted with a large mesh, second with the intermediate and the third with the smallest mesh screen, the height of the shoe above the mesh being adjusted accordingly. The use of this method of operation considerably decreased damage to the testa and to the kernels. It was found necessary to keep the nuts properly stored to ensure that the shells were crisp and friable, thus rendering decortication an easy operation. With one of the standard machines fitted with a feeder 140 kg of Red Valencia ground nuts could be decorticated in one hour.

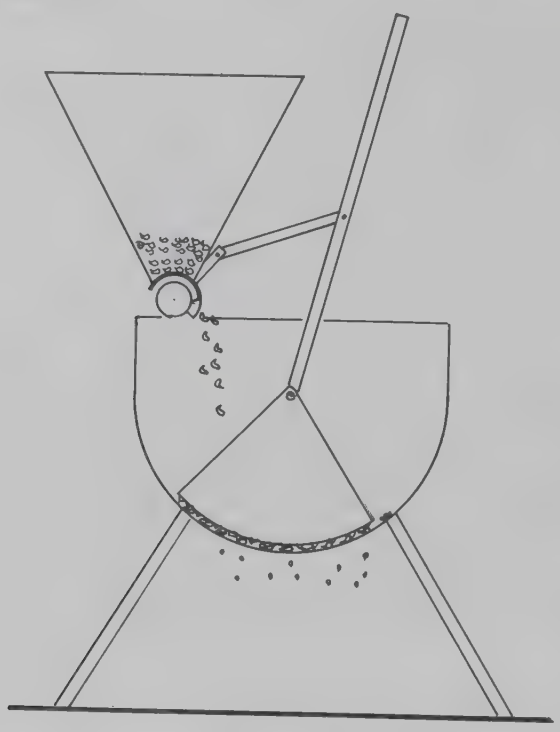


Fig. 2.– Schematic arrangement of standard hand operated groundnut decorticator with liked feeding attachment.

RESULTS AND DISCUSSION

This work has shown the considerable engineering difference in approach required when decorticating tropical nuts with though leathery shells and fragile kernels, and friable shells containing kernels with a delicate testa which must not be damaged. It demonstrates that in every case one of the most important aspects is to ensure that preprocessing is given the greatest attention; unless this is done optimum efficiencies cannot be obtained.

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Session 8b

Summary and Conclusions

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It was suggested at the beginning of the session one could subdivide this subject according to several objectives as follows.

I) The preparation of special processed and composite foods for the vulnerable groups in the population (also ingredients for these).

II) The improvement in quality, attractiveness, palatability and availability of existing foods used by the population at large.

III) Easing the preparation, saving in many instances energy, and providing the development of convenience forms of existing foods.

IV) The development of new foods or the enrichment of widely consumed ones, utilizing locally available materials.

V) The saving of imports by the use of local foods.

A main conclusion of the meeting was that the development of food science and technology program needed well formulated policies from developing country governments if successful commercialization and utilization of the products were to be achieved. The *technology* of course, plays a vital role and in many cases this is now well developed and available; but the application of that *technology* remains very difficult to realize in many instances.

Papers presented described the possibilities of producing high calorie/protein types of foods, using raw materials available in various developing countries. It was indicated that there was no absolute shortage of protein for meeting the needs of the vulnerable groups. Vegetable protein from various sources was available in most developing countries including particularly a number of oil seeds, like peanuts, sesame or soybeans.

Note was also taken that foreign food products were being adapted in many countries, a particular instance being bread which was growing in importance.

Account was given of bread and other wheat products being produced by incorporating a proportion of local protein and starches to save on imported wheat.

It was mentioned that due to a number of factors, a world shortage of wheat, cereals and green legumes of about 85.000.000 tons was feared by 1985, and many countries would then be forced to rely on the effort of its science and technology to avoid starvation of its population.

Application of research findings is necessary, and it will be essential to alert top government officials as to the nature of the problem and the advance planning and development that will be necessary. In such steps the fullest possible use of the locally available raw materials should be envisaged. Processing, storage, marketing, acceptability and costs all need to be taken into the planning considerations.

The final conclusion was that a lot of effort had been spent by many nations in order to solve the scientific and technological problems involved in making greater use of local raw materials. Given the necessary finance and the political will, this could be converted into large scale operations to avoid future deprivation and distress.

Round-Table 7

COOPERATION BETWEEN RESEARCH CENTRES AND INTERNATIONAL ORGANIZATIONS FOR TRANSFER OF TECHNOLOGY TO DEVELOP FOOD INDUSTRIES

Chaired by

H. A. B. Parpia, Italy

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H.A.B. Parpia

It is my pleasure to welcome you this morning to the Round Table discussion on Technology Transfer to develop food and agricultural products processing industries. I am thankful to the organizers, particularly Prof. E. PRIMO and Dr. SALVADOR BARBER for asking me to Chair the Round Table. At the outset I must express my gratitude to all the eminent personalities who are members of this Round Table for accepting the invitation to participate and to cover various important aspects of the subject. I also express my thanks to the audience for the manner in which they have responded. Besides the members of the panel, we wish to encourage participation from the floor of several individual scientists, technologists, industrialists, management specialists and others.

To introduce the subject, I would like to say only a few words. Food technology can make a real impact on the solution of the world food problem if it can be utilized effectively to conserve and process food. Even if half the losses which occur at post-harvest level can be prevented most of the developing countries would become self-sufficient. Besides, in the process of preventing the losses, we have to develop industries where the technologies have to be used. This would generate employment and raise income levels so that people can buy the food. This would also stimulate agricultural development by assuring better market for the producers and provide them with resources to use greater inputs for increasing yields.

The transfer of technologies and their use depends upon the competence of men and the institutional facilities available for this purpose. Therefore, training of manpower; building and management of institutions for identification, modification, development and use of technologies is fundamental to the transfer of appropriate technologies and for achieving technological self-reliance.

The economy of developing countries are agricultural, if full benefits of production are to be derived proper use of post-harvest technologies is not only important, but urgent. This would in fact accelerate the development of their economies as a whole.

Before I invite the first speaker, I wish to give a brief definition of post-harvest technology. It is an inter-disciplinary use of science after a crop or an animal has been raised and is concerned with all aspects of handling, storage, milling, processing, management, marketing and distribution to meet the food and nutrition needs of the consumer.

As the subject on which the members of the panel are to speak are interrelated, I would suggest that we request all the speakers to complete their presentations first and then have a discussion so that we have in front of us a proper perspective.

TRANSFER OF TECHNOLOGY FROM ADVANCED TO DEVELOPING COUNTRIES, AFTER SUITABLE ADAPTATION AND ALTERATION*

George F. Stewart

University of California. Davis, USA.

Since this is supposed to be a round table discussion I would, at this time, like to just present some general observations. Hopefully these remarks will stimulate some meaningful discussion and raise some important and relevant questions.

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*Text provided by the author.

There are some rather general considerations concerning technology transfer from developed to developing countries that determine success or failure. My experience with, and analysis of the problem suggest that the more important factors are: 1) Recipient nation policies and practices regarding industrial development, especially the food processing industry, 2) Availability of capital investment and foreign exchange, 3) Appropriate infra-structures in recipient country, especially distribution and marketing facilities and services, 4) Need for, and appropriateness of technology to the recipient country, 5) Raw materials availability for processed foods to be produced by new technology, and 6) Ability for suitable adaptation and modification of new technology in developing country.

Many scientists and technologists, like any other human beings, may be easily tempted to take on a technical assistance assignment overseas, especially if he or she has never visited a particular country before. All too often, having asked no questions, he or she finds himself (herself) in a foreign country trying to help with the development of that country's food industry when there is little or no chance of success. So, first of all, I would implore the would-be helpers to ask some key questions before saying, "Sure I'd be glad to serve'. The chances of success would be ever so much greater.

Perusing the list of considerations just related above might suggest to the technical aid person that there is little he can do about the first three or four items, as they deal with the economics, sociological and political aspects of the "aid" problem. I say that, notwithstanding the food scientist involved needs to find out if the necessary conditions exist in the recipient country for success. This may mean going to fairly high levels of government and industry to find out. So be it! .

Before you can successfully introduce a new technology to a developing country there must be government policies and practices that favor the development of a commercial food processing industry. These policies and practices are critical as they affect credits and banking, marketing and distribution services, wages and employment, export-import regulations, food standards and grades programs, food safety, nutrition education, etc., etc. Without a positive attitude and programs to develop a food processing industry little or nothing may happen, even if you have the greatest process for preserving food in the world.

Similarly, there must be money available to build and equip plants to apply the new technology also and in many cases foreign exchange to import machinery. The latter a critical consideration at this period in time when most developing countries are very short of foreign exchange and where the processing industry does not have a high priority in gaining access to any foreign exchange.

No country can have a flourishing food processing industry unless it possesses the requisite infra-structures, especially a modern distribution and marketing system. In many developing countries there are only primitive systems that are ill-adapted to distributing and marketing processed foods, especially for those requiring some care (e.g. refrigerated foods).

There should be a real need for a new technology in developing country and it ought to be appropriate to its stated development plans. Processed foods can be produced for export and/or for local production and it should be clear which market (either one or both) the new technology will cater to. An example of a particularly inappropriate technology for developing countries, in my opinion, is radiation preservation. This has been impressed on me several times as I have seen in various parts of the world considerable sums of money and a lot of effort expended on a transfer of this technology. A sheer waste, in my opinion.

Raw materials availability or potential availability is a critical problem in adapting a new technology. Not only is this true of the food raw materials, but also other ingredients, and packaging and other materials. Importing such material may be prohibitive on account of foreign exchange problems. Many examples of technology transfer failures could be cited in support of this statement.

Considerable attention should also be paid to providing opportunities for adaptation and modification of a technology being transferred to a developing country. It is well to remember that labor is readily available and at reasonable cost in these countries. Thus, labor-saving components of a technology are not of much importance in the transfer. Opportunity should provide for local adaptation and modification which take account of this fact. Too many technologies have been exported as "turn key" affairs with little or no opportunity for local inputs.

One last observation – technology transfers for agricultural enterprises are generally easier to accomplish than in food processing and preservation. I have a specific experience in mind where we were industrializing the poultry industry for certain Southeastern Asia countries. It proved fairly easy to commercialize the broiler and egg industries of the area where local adaptations and modifications succeeded admirably. However, we were not able to succeed so well in introducing processed egg products and processed poultry meat industries. The problems of sophistication were too great to solve and the marketing and distribution system was not adequate for developing these products through channels of commerce.

H.A.B. Parpia

Thank you very much, Professor STEWART. I think you have given the right direction and stimulus to the entire topic which should be of inmense value. To follow up, our next speaker is DR. AGIDE GORGATTI NETTO, Director, Instituto de Tecnología de Alimentos, Brazil.

Agide Gorgatti

The subject of my speach is the transfer of technology between developing countries and research requirements.

I would like to present some ideas and say something about our experiences in Brazil.

I believe that most of the developing countries have been trying to adapt technologies from developed countries and also to produce their own technology to solve their particular problems. As we all know, there are many differences between the problems that are to be solved in a developed country and in a developing country. And the most difficult point is to establish a scale of priorities in order to know exactly which are our more important needs in relation to the technologies we need for the preservation and production of new products or, for example, to improve the quality of a commercial product. When we are trying to import as a first step and to adapt the imported technology we are also importing or adapting cultural aspects that are a part of such a technology. I think this is a major point that we should discuss here. When we are trying to bring some consultants or specialists, when we are trying to send people abroad to study, we should conduct at the same time a research programme in order to know the measures to be taken to plan a systematic transfer related to the cultural aspects that can come with it.

Another important point is the need to develop programmes in a very integrated way, considering raw material production aspects, processing technologies, marketing and so on. This only can be done assuming that changes can be made within the country. Many institutions in developing countries are working in isolation from each other and efforts are not combined to solve specific problems. To overcome this problem, there is a need for people who are concerned with the development of technology to increase cooperation and to show the government how a better use of the human and financial resources can be made to develop a research programme. We usually have money available to develop only a certain number of projects and therefore, we should think how to optimize its use through development of a integral programme of work.

The best way to carry out the transfer of technology between developing countries is to try to invest as much as possible in the improvement of the professional capability

of researchers, enabling them to do research work of interest to their country at the place where they receive training. They should bring their problems and try to solve them in an adequate way. This raises the problem of getting a technology. Professionals from developing countries receive in advanced countries the kind of training that they are not able to use after coming back to their own countries. I think it is necessary to attach scientists to a specific project, the objectives of which must be to develop a certain type of technologies of interest to the country to which they belong.

The transfer of technology between developing countries, I believe, should be easy, for example among the Latin American countries, as their problems are similar and there are no language barriers. This facilitates the communication and also the transfer of technology.

Another point to be considered is the need for action to solve the specific and most difficult poblems. One of them is to avoid the losses that occur after harvesting. All the benefits of increasing production by developing new varieties is lost through insects, rodents and so on. The Government in our country is emphasizing the need for an institution to expend at least 30 per cent of its capability for the integrated development of projects that can solve this problem. For this a three way cooperation of the institution, the government and the industry is essential. In some cases, where such cooperation has been established the effort has been very successful. It also provides a kind of transfer, at least of know-how, between the industry and the institutions.

These are the most relevant points of my formal presentation. Thank you.

H.A.B. Parpia

DR. GORGATTI NETTO has added some very valuable points. I just want to make a little comment, because he raised the vital issue regarding technology not being isolated from culture. This is the key point in the transfer of technology, not only among developing countries but also among developed countries and for transfer from region to region. By regions I do not mean political regions of Latin America or Asia, but geographical and ecological areas, for example the transfer of technologies between the tropical countries where the storage problem to which he referred are similar. The magnitude of this problem of losses is large and cooperation between developing countries for the exchange of technology on the subject can prove valuable.

Now, it is my privilege to call upon DR. K.T. ACHAYA. He will speak on the subject of research and development in the field of food science and technology in developing countries and the transfer of results to industry, because the transfer problems here in developing countries are very different from those in advanced countries.

RESEARCH AND DEVELOPMENT IN THE FIELD OF FOOD SCIENCE AND TECHNOLOGY IN DEVELOPING COUNTRIES AND TRANSFER OF RESULTS TO INDUSTRY*

K. T. Achaya

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In any consideration of the broad problem of research and development, it is convenient to assume certain premises. In this instance, the assumptions are that the laboratory is situated in a developing country, that it is concerned with the area of food, and that its purpose is to develop food products or processes for adoption by industry. If basic research and its place in a technological laboratory are put aside for the present, the topic chosen resolves itself into three broad areas: Research, Development, and Transfer to Industry. These will now be considered in order.

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*Text provided by the author.

Research

It many seem obvious that the choice of a problem should be conditioned by the needs of the food industry of the country. Yet when a bright young scientist joins a research laboratory, he will often insist on sticking to the kind of work which he has done for his Ph.D.; this is understandable, since in dealing with something familiar, he feels confident of not falling flat on his face. Even later in his career it is not at all uncommon to find him choosing problems which are fashionable in the literature currently emanating from the advanced countries, whether they are relevant to his own environment or not. As a small example, a lot of work is being done in various home science institutions in India and elsewhere on canning and bottling, even though these preservation methods are and will continue to be of really minor importance in these countries for economic reasons. On the other hand traditional preservation methods like salting, pickling and fermentation, which offer a wealth of possibilities for both basic and technologically-oriented examination, are just ignored. It is important to critically study and survey in depth local food systems and practices so as to identify matters which are of real practical and economic importance. The watchword should be: don't manufacture problems, find and identify them.

Having taken up a subject for study, speed of solution is almost always important. It seems best to set up various stages towards solution, and a small quantum of attainable targets for each stage, rather than to speak in terms of a total solution with every i dotted and every t crossed. By the time the solution arrives it may be too late to be of value. Specific measurable achievements, even if small, encourage clear and stepwise thinking.

This leads in turn to such questions as the size of a laboratory and the strength of its staff. Size in the developing countries is all too often mistaken for strength and substance. It is so easy for a laboratory to grow flabby and amorphous, and thus to lose its muscular flexibility. In fact problem-solving-ability is quite often inversely proportional to size. People are certainly needed to solve a multitude of problems. But when they proliferate without check, effectiveness of supervision and direction inevitably begin to suffer. With staff increase, a corresponding expansion of utilities does not usually occur, and often is not even possible – things like physical working space and working volume, chemicals, equipment, and such technical services as engineering or instrumentation. Moreover since staff migration from any growing laboratory in a less developed country is very low for lack of opportunity, the same staff grow in age and status and inevitably move up from the bench or the pilot plant to a chair, leading to a shortage of those working hands who will actually deliver the goods.

When the problem chosen is likely to be a large and important one, it is useful right from the start to have teams of workers drawn from several disciplines. This ensures that all members of the team are involved in the project from the beginning. Enthusiasm must be sustained by frequent team consultation and by such apparently simple devices as sending all reports to all members of the team so that nobody feels left out and superfluous. In the earlier stages of the project, the laboratory men usually play the more important role; later in development it is chemical engineers and mechanical engineers who usually have to take the lead. Personal pride and jealousies must be carefully watched by the director of the laboratory before the canker kills the rose. On the other hand, it would be unwise, even were it possible, for the director to be present personally at all project meetings, since this always weakens initiative and leadership within the team. The rung of the hierarchy just below the director could have a big role to play here.

Pilot–plant development

When laboratory work has been promising, and when industrial development seems attractive, the next stage is usually to do everything on a distinctly bigger scale in a

pilot plant so that snags which do not show up in laboratory work, such as a filtration problem or a heat transfer problem, can be assessed and overcome, and more realistic cost data obtained. It is better to be ruthless in making a decision at this point whether to go in for pilot plant work, since it is expensive. One way of assessment is to have an evaluation of the laboratory process by an independent person or group, who must be encouraged to play the role of devil's advocate, even though this will not exactly make for popularity.

As a rule-of-thumb it may be accepted that for a pilot plant to be meaningful, it should be one-tenth to one-fifth the likely eventual commercial size. In developing countries a case could be made out in certain instances for large, fully-fashioned, almost semi-production units for pilot plant work: for example, the product so made could be consumer-tested or even test-marketed so as to reduce the eventual entrepreneurial risk. On the whole however it is better in the food field to stick to smaller sizes of pilot plants. In buying equipment therefore it is essential to seek flexibility of use; one needs to think well ahead when planning to buy pilot-scale equipment, since often it takes a year or two for a unit to arrive. Use of improvised equipment must always be encouraged; innovative skill leads to confidence, and to basic design knowledge which can be banked and drawn upon in future. Indeed the designing of equipment is excellent training for engineers and chemical engineers, who then become the nucleus of expert design groups through which to serve the needs of the laboratory and the industry of the country.

The involvement of an industrial outfit in pilot-plant development is an invaluable asset. While the laboratory runs some risk of the charge of being called exclusive ("why them, why not us? "), the chances of commercial success are very much better for such participation. It is also important to have clear objectives even for pilot plants and not to keep on working them indefinitely and aimlessly once the bugs have been ironed out, and the process, design or cost data gathered. Such endless working is a real danger especially where the food output from a pilot plant builds up for itself a small but dependent clientele, who then become the sole but really irrelevant reason for pursuing the operation.

It is often essential for Government to set up a separate funding agency which provides risk capital, drawn perhaps from a cess on industrial production, for such large-scale development. There is the further advantage that such an agency will naturally require an independent evaluation of a laboratory process or product before deciding to invest money. A further possibility is to obtain development funds for pilot plant work from an industry or group of industries, which will be paid back to them (perhaps in part) by the risk-capital agency if the pilot plant does not fulfil the objectives which are clearly set out for it by all parties concerned at the time the funds are invested. In fact, the entire phase of pilot-plant development, instead of being accepted without question as an essential scale-up for all work that is done in the laboratory, should be treated as an opportunity for a rigorous examination of the objectives, appropriateness, feasibility and commercialisation of the entire project.

Transfer to industry

The last step in the chain is the transfer to industry of a pilot-plant development judged to be potentially successful both by the laboratory itself and by a prospective entrepreneur. How far does the laboratory go in effecting this transfer? The possibilities range all the way from a turnkey job to simply the furnishing of written reports and design data which are then to be further engineered, fabricated and put into operation by the entrepreneur. At the start, a laboratory would do well to establish its credentials with a couple of turnkey jobs. Not only will this raise the laboratory's standing with industry, but it will create tremendous self-confidence in the laboratory staff that they can deliver the goods. Turnkey jobs are of course very agreeable to industry, which is

quite content to let the laboratory take all the sweat of the operation. But such total jobs take up a lot of staff time and effort, usually to the detriment of other projects. As the occupations of the laboratory grow, it is advisable to encourage in the less-developed countries the formation of firms of consulting engineers who can be entrusted with erection jobs based on projects or designs developed in the laboratory. Eventually even the designing of laboratory processes by outside firms may be possible with mutual trust; such sharing of labour and expertise will be to the advantage of both.

H.A.B. Parpia

We have a colleague, who has been handling international development research problems on a worldwide scale from Canada, MR. J.H. HULSE. I am sure he has an important contribution to make.

RESEARCH AND DEVELOPMENT IN ADVANCED COUNTRIES AND TRANSFER OF RESULTS FOR INDUSTRIAL DEVELOPMENT TO DEVELOPING COUNTRIES*

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The term "transfer of technology" has come to mean the transportation of a technological device or system from one place to another. In recent years it has been much concerned with the transfers of technological ideas and processes between the more developed and the less developed nations of the world.

It has been estimated that, in terms of total cost, more than 95 % of the world's research is carried out in North America and other developed countries and less than 5 % in all of the LDCs together. Considering the relative importance of food processing and distribution to the economies of the developed countries, one might expect to find significant transfers of useful food technologies from the richer to the poorer nations. In actual fact, the contribution of the developed countries to the less developed countries of practically useful systems of food transformation, preservation and distribution appear to be comparatively meagre and in many instances the technological transfers which have been attempted have proved inappropriate to the needs and conditions of the developing nations.

Within this brief paper it might be useful to try to explore some of the reasons why technological transfers have in the past failed and how more successful cooperation between the technologists of the more developed and less developed countries might be encouraged in the future. The subject will be discussed under four headings: 1) general philosophy and level of mutual understanding; 2) education and training; 3) research systems; and 4) direct industrial

1. General Philosophy and Mutual Understanding

There is a growing body of circumstantial evidence to suggest that the majority of scientists and technologists and scientific institutions in developed countries possess a very inadequate knowledge and awareness of the priorities, problems and conditions of developing countries. The many failures of attempted transfers of technology bear witness to the difficulty of transferring technologies between countries widely different in cultural heritage, technical and economic development. There is also an apparent tendency in developed countries to regard all less developed countries as a single block: "the third world", and to assume that they all suffer from identical problems and more or less equal levels of poverty and malnutrition. It does not appear to be recognized that

*Text provided by the author.

greater differences in economic, social and technical development are to be found among the so-called less developed nations than between some of the less developed and those classified as "developed". India includes among its citizens a far larger number of university graduates in food and related sciences than are to be found in many socalled developed countries. The Government of India supports what is probably the largest food research institute in the world. The Universities of Egypt were teaching science related to food and agriculture long before the first university was established in North America.

Much of the ill-considered advice given to the developing countries by so-called "experts" from the developed appears to be based on the assumption that what is good for developed nations must essentially be good for the less developed. It presumes that the philosophies which guide food research and development in North America and Europe are valid for the struggling economies of many African, Asian and Latin American countries.

Industrial food research in developed economies in North America and Europe is predominantly concerned with, first, the creation of new products and processes, and second, with quality control. Both product and process research and development are aimed primarily at saving labour. The growing spectrum of "convenience" foods, in the form of canned and frozen, pre-cooked meals, and prepared mixes of soups, sauces breads, cakes and desserts, are intended to reduce the work load of the industrial cook and the housewife in their respective kitchens. An "improved" process of manufacture invariably means a process which generates a larger number of products per man hour of labour. Since laboratory technicians, in common with other components of the labour force, demand higher salaries every year, the motivation in quality control research is largely to replace human effort by mechanized and automated analytical instruments of ever increasing complexity of design requiring comparatively little human effort per unit of output.

Furthermore, in the developed countries where transportation facilities, including facilities for the distribution of frozen materials, are readily available and where economies of scale in production are demonstrable, the trend is towards large centralized, highly mechanized, manufacturing units with a large output per unit of labour.

Such a philosophy is quite inconsistent with the needs of many developing countries where it is important to provide gainful employment for as many people as possible and where in general, labour is comparatively inexpensive. Consequently, many of the technologies which are prevalent in developed countries are ill-suited in scale and design to the needs of the less developed.

Furthermore, highly mechanized systems, whether it be of production machinery or laboratory instruments, demand ready access to an efficient source of maintenance including spare parts. It would be interesting but probably distressing to produce an inventory of the number of instruments and machines which have been imported from developed into developing countries which are laying idle for want of adequate maintenance and repair facilities. Large centrailized manufacturing units are out of the question in those countries where road and rail transportation is inadequate to ensure reliable deliveries from farm to factory and from factory to the market place.

Finally, and briefly, one might refer to the philosophy which controls the manner in which bilateral and multilateral aid is offered by the developed to the less developed countries. Since much of the aid is tied to the goods and services of the donor country, there has been a strong tendency to devise aid programs, whether in the form of production facilities or research laboratories, which make maximum use of machines and instruments from the donor nation(s). In these circumstances, the program of work tends to be dictated by the machinery and equipment available rather than the equipment being chosen and designed to suit the needs of the optimum program.

2. Education and Training

Since this is a subject of special concern at this conference, it will be referred to only briefly here. Nevertheless, in spite of the many words written and spoken on the subject of training being appropriate to needs, one still encounters graduate students in the universities of North America and Europe carrying out research on thesis subjects which appear to bear little relevance to the priorities of their own national economic environment. Many of these graduate research programs are inapplicable both in scientific content and in the methodology and techniques involved. It is exceedingly wasteful of limited human scientific resources to train young scientists and technologists from developing countries in techniques which require equipment that their countries can neither afford to purchase nor to maintain. Similarly to direct their intellectual energies to technically and economically unsuitable projects such as the production of microbial protein and fish protein concentrates rather than to simple and applicable methods of protecting, preserving and utilizing their indigenous food sources, appears to me not only mindless but rather cynical. Unfortunately, the criteria appear to be dictated by what is ingenious and what will be acceptable to a referred scientific journal rather than what is likely to prove practically useful when the graduate returns to his or her own country. It is gratifying to observe that some agencies now encourage graduate students to receive their theoretical training in a university in a developed country, where such training is not available in their own country, and to return to their own country to carry out their practical research on a problem which is directly relevant to their nation's need. This appears to be eminently sensible in that I believe it can be proposed that scientific principles are more readily transferable between different socio-economic systems than are technologies.

3. Research Systems

Throughout the developed countries one finds many institutes of food research which, though they vary in detail, tend to have been created from the same basic principles. These institutes, whether they are owned and operated by private industry or governments, or some mixture of the two, usually include product and process development laboratories, and pilot plants together with highly instrumentalized, analytical, physical and microbiological laboratories where standards of product identity and quality and process control are defined and tested.

For some of the reasons I have already cited, one finds a growing number of such institutes in developing countries. Many of these are the result of advice from foreign experts, provided by bilateral or multilateral aid agencies. In fact, where these research institutes were equipped through multilateral or bilateral aid, the recipient governments of the developing countries were often pressed to accept a large inventory of scientific and pilot plant equipment often before the needs of the local industries, and therefore the most relevant program of research, had been defined. Under such circumstances, the program of research has tended to be selected and justified more by the nature of the equipment provided than by the immediate needs of the indigenous food and agricultural economies and processing industries. The creation of such highly equipped institutions inclines the native food scientists and technologists to remain inside their laboratories and pilot plants, standardizing and learning how to operate the equipment, rather than to working with and helping solve the problems of the existing food processing and distributing industries. Furthermore, after the aid funds dry up, the developing countries find themselves left with a very expensive institution which constitutes a drain on foreign exchange reserves in order to maintain and replace the instruments, devices and machinery originally installed.

It would seem more logical to assist the scientists of developing countries to spend more time working with their indigenous food industries, learning what are their problems and their opportunities for improvement before embarking upon the definition of a

research program and the design and construction of an expensive research institution. It is my belief that the small indigenous industries of the developing countries would benefit far more from technical assistance and advice which would enable them to operate more efficiently the machinery and equipment they already possess than being offered new products and processes demanding of capital investment they often cannot afford. Furthermore, foreign "advisers" and "experts" might do well to spend more time listening than advising the people of the developing countries who generally are far better aware of their own problems than are those from other countries. Most important, we should all try to avoid the danger of carrying a solution which is looking for a problem. In the past, too little time has been devoted to thoroughly understanding the problems of food preservation and distribution in developing countries, particularly the economic, social and logistic aspects of the problem, before offering a technological remedy.

Also, too little opportunity has been afforded to the scientists and technologists of developing countries to meet together and solve their own problems. One extremely interesting development has taken place in South and Southeast Asia where the food scientists of that region have created a working group which has come together on several occasions and has defined problems of common interest to the whole region and is now developing a series of networks of projects related to the principal problems, each project being complementary to the needs of the whole region. It is suggested that much greater encouragement and support should be given to such cooperative effort in which scientists and technologists from a region meet together frequently and develop a cooperative research and development program directly relevant to the needs of all of their countries. The Southeast Asian group, in addition to its other activities have produced an interesting directory which lists and classifies all of the research in food science and technology which is in progress throughout the region. It is also establishing a technical information and technical literature service throughout the region.

4. Direct Industrial Transfers

Many developing countries have sought to import processing technologies through licencing agreements with food processing companies in developed countries. In addition to the difficulties of transplanting total technological systems or components of such systems into an unfamiliar and uncongenial environment, the direct cost of technology transfers may prove exceedingly burdensome to recipient developing countries.

The direct costs of technology transfer include the right to use patents, licences, manufacturing and marketing know how, trademarks, advertising materials and technical support services. An UNCTAD study in 1971 estimated that for all developing countries during 1968 the direct cost of technology transfer amounted to $1.5 billion in foreign exchange and that direct transfer costs to developing countries are increasing at the rate of roughly 20 % per annum. If this trend is extrapolated in constant dollars, the result will be a net foreign exchange debit to the developing countries of nearly $9 billion by 1980.

Governments of LDCs which enter into technology transfer agreements may find themselves bearing a heavy burden of indirect costs, including the deliberate over-pricing of raw materials, capital goods and equipment sold by the parent in a developed country to its subsidiaries or licencees in less developed countries. Such deliberate over-pricing is a means by which the parent company can quickly repatriate its foreign investment. For the developing country it represents an extremely heavy drain on its foreign exchange resources.

Many such licencing contracts include restrictive clauses. In a study of the five countries of the Andean Pact it was discovered that more than 70 % of such licencing contracts between food and beverage companies in that region and related companies in developed countries prevented exports of the products manufactured by the Latin American licencees.

There have been many sorry instances in which developing countries have been persuaded by foreign experts to purchase and install food processing facilities quite unsuited to their needs. It is obviously necessary for the developing countries to increase their own technical expertise and resources in order to be able to make the best possible choices among the alternative technologies available to them and also to recognize when none of the processing systems commercially available is ideally suited to their needs. While it may be many years before every developing country can have within its own boundaries all of the expertise it needs, there is here again, I believe, an opportunity for developing nations to cooperate with one another and to establish regional research and technical advisory services so that Asians are advising Asians, Africans are advising Africans and that the developing countries of these regions are not entirely dependent upon the expatriates of the more developed nations.

In summary, I would urge that every encouragement be given to systems of research, technological development and adaptation which enable groups of developing countries to cooperate together to solve their common problems and to realize their common opportunities; that governments of developing countries seek to become less dependent upon the more developed countries both for technological transfers and advice; and that multilateral and bilateral programs of technological assistance be administered in less restrictive terms and that adequate resources and time be assigned to permit clear and comprehensive definition of technological needs before any solutions, particularly those which may involve costly technological transfers, are recommended.

H.A.B. Parpia

I cannot help but make two comments in the light of what MR. HULSE has just said. Some time ago, as the Director of the Central Food Technological Research Institute in India, some of my colleagues advised me to purchase rice milling equipment for their use. We examined the problem and decided to discuss it with a rice miller of the City. We asked one of the millers to give us his mill or rent it so that improvements could be *made on it* under actual commercial conditions. My colleagues accepted this proposal. The mill was taken over and the improvements made were of real value to the industry. This is how the gap between research and industry can be filled. The work was carried out as a part of industry. I would also like to emphasize Mr. Hulse's point of view that the cost of labour in developing countries can be as low as one third to one sixth of what it is in advanced countries and therefore, the technologies should be employment generating and capital saving yet scientifically modern to produce products of quality.

May I now request MR. HULSE to take the Chair as it is my turn to speak on the role of international organizations in transfer of technology to develop food and agricultural products processing industries.

At the beginning of the discussions, I emphasized the importance of postharvest technologies in accelerating the development of economies of the developing countries which are essentially agricultural Between 60–90 per cent of their Gross National Product comes from agriculture. Developing countries account for approximately 7 per cent of the world' industrial production. If this situation has to be changed, one of the most effective means of doing so would be to build food and agricultural products processing industries. These industries would require many other raw materials besides the agricultural produce. They would need containers, packaging materials, fine chemicals, etc. Already, it is well known that agricultural processing industries generate the largest chain of employment. Agriculture alone, as a means of employment in rural areas of developing countries, has reached a saturation point. Therefore alternative employment has become an urgent necessity.

Recognition of the problem of developing countries for greater self-reliance is not enough by itself unless ways and means can be found to assist them in making progress

through their own efforts. This is where the international agencies, particularly FAO, can play an important role.

As a first step, in 1971 FAO identified the following areas and sub-areas where the developing countries needed assistance:

A.– Food Grain Industry

Food grains constitute nearly 80 per cent of the diets of a large majority of the people and are also the largest single source of calories and proteins in the world. They include wheat, rice, corn, sorghum, millets and a number of grain legumes such as the chick pea (Bengal grass), mung beans, etc. If their quantitative losses can be prevented and quality maintained through proper storage, handling and processing, it will help many countries to achieve self-sufficiency in their nutritional requirements. Major areas of food grain industry which require inmediate attention are:

1. Storage and handling of food grains. This should include design of suitable structures and development of post harvest drying, handling and infestation control techniques for use in rural and urban areas.
2. Moderni zation of milling and processing of food grains.
3. Manufacture of cereal products modern and traditional including baking industry.
4. Starch and starch derivatives.
5. Manufacture of food grain by-products and waste utilization.

B.– Nuts, Oilseeds and Vegetable Protein Industry

Nuts and oilseeds are not only the main sources of edible fats and oils in which many developing countries are deficient but also the second largest potential supply of proteins after food grains. The traditional concept of oil millers must change and they should realize that the industry must also become a protein industry. With the modernization of the entire traditional operations specially in rural areas the quality and quantity of oil extracted can be increased substantially through the use of solvent extraction. With proper control of quality and processing conditions, the extracted meal can retain high biological value and can be used for human consumption in a number of food preparations. Better use of oilseed meal can also increase the total available supplies of food for human consumption. Exotic nuts such as cashews are large foreign exchange earners for many countries. Main areas of the industry which deserve priority attention are:

1. Oil extraction from oilseeds and food grain wastes
2. Processing of oils and fats for various end uses
3. Manufacture of high protein oilseed meals, concentrates, isolates
4. Protein foods using oilseed proteins
5. Better use of non traditional sources of oils for industrial purposes, such as soaps, lubricants, etc.
6. Improved processing of exotic nuts to manufacture better quality products.
7. Manufacture of by-products.

C. Fruit and Vegetable Processing Industry

In many developing countries the losses of fresh fruits, vegetables, roots and tubers are very high, amounting to 25 per cent or more. If the products can be properly handled, prepackaged, stored, transported, processed and marketed, the food supplies can be increased substantially and economic returns of the growers improved, besides serving the consumer better and earning foreign exchange. The areas of this industry which need attention are:

1. Picking, transport, waxing, grading, packaging, cold storage and transport of fruits vegetables for market.
2. Drying, freezing, canning and concentration of pickling, salting, curing of fruits and vegetable products.
3. Utilization of waste for by-products manufacture.

D. Starch and Sugar Industry

The prices of sugar and starches are very high in most of the developing countries. Besides the high price of raw materials, the other factors which add to the price are uneconomic operation and improper utilization of waste for the manufacture of by-products in many cases. The areas of this industry which require attention are:

1. Manufacture of sugar from beets and sugar cane.
2. Manufacture of high quality starch and glucose from certain cereals and tubers.
3. By-product utilization in composite animal food formulations.

E. Fermentation Industry

Fermentation as a means of food preservation is one of the oldest known technologies. There are many traditional fermented foods consumed in different parts of the world. With economic development and urbanization, the demand for commercial production of these foods offers good potential. This calls for modernization of the industry and also for establishing manufacture of a number of modern fermented food products. The areas of this industry which require attention are:

1. Wine
2. Brewery
3. Distillation
4. Yeast manufacture
5. Amino-acids and glutamates
6. Traditional foods, such as tofu, shoya, tempeh and sauces.

F. Non-alcoholic Stimulant Products.

Items like tea, coffee, cocoa and certain traditional drinks in different parts of the world, are consumed in large quantities. These products are also major earners of foreign exchange for them, but the industry faces a number of difficulties as the changing pattern of consumption requires new products to fill the social and nutritional needs. The areas of this industry requiring attention are:

1. Tea, packaging, processing, storage and transport
2. Coffee, packaging, processing and transport
3. Cocoa, storage and handling
4. Chocolate and confectionary products manufacture
5. By-products such as caffine and other products.

G. Spices and Essential Oils Industry

Although these products are large foreign exchange earners, they are exported by many countries as raw materials and there are many problems about maintaining quality standards. If maintenance of quality standards can be improved and the products are exported as finished spices, oils and extractives, the foreign exchange earnings can be increased substantially, besides providing additional employment. The areas of this industry which deserve priority are:

1. Pre-processing and packing of spices for home market and export.
2. Manufacture of spice oil and extractives.
3. Manufacture of by-products.

H. Meat and Poultry Industry

This is one of the industries which deserves urgent attention as the conditions of abattoir, handling, packaging, processing, distribution and marketing leave much to be desired in most of the developing countries and can also prove a serious health hazard. Its modernization can be of great value for providing stimulus to development of animal husbandry. Priority areas of the industry are:

1. Abattoir, packing, storage and marketing of fresh meat and poultry.
2. Curing, canning and freezing of meat and poultry products.
3. Collection, cleaning, grading, coating, packing, storage and distribution of eggs. Manufacture of egg powder.
4. Processing of hides and skins.
5. Manufacture of leather goods on cottage and small scale.

I. Milk and Dairy Products

Milk and its products continue to occupy a high position in the nutrition of most countries including the developing ones, in spite of their high price. Considerable amount of attention has been given to its development in a number of countries and the industry deserves continued encouragement and support. The areas which need emphasis for further development are:

1. Market milk – its collection, standardization, processing and distribution.
2. Manufacture of milk products, such as weaning and infant foods, cheese, butter and other dehydrated and concentrated products.
3. Better utilization of by-products such as casein and whey.

J. Fish and Fish Products

The potential for the development of this industry in most of the less advanced countries is quite large. Their fish resources have only been exploited to a limited extent. Fish cannot only improve the diet and raise nutritional standards with regard to high quality protein, but can earn larger amounts of foreign exchange in many cases. The areas of fish industry which deserve inmediate attention for further improvement and modernization are:

1. Collection, handling and packaging for fresh market, cold storage, freezing, smoking and refrigerated transport.
2. Canning, curing, pickling and drying.
3. Fish by-products.

K. Animal Food Industry

The need for animal feed is being increasingly felt in many countries of the world to increase production of milk and other animal foods. The areas which deserve urgent attention are:

1. Manufacture of balanced composite poultry and animal feeds from the available raw materials such as animal by-products, fish meal and vegetable protein.
2. Manufacture of pet food.

L. Natural Fibre Industry

One of the largest areas of agro-industries is the manufacture of products from natural fibres. The products from the fibre industries represent culture, tradition, art and means of livelihood for a substantial portion of the world population besides meeting their clothing, packaging and other needs. For its survival and future progress it is most essential to base the industry on a modern scientific foundation. This will provide impetus to the raw material production. Natural fibres can be used to produce high quality fabrics by mixing them with artificial fibres. This will supply exotic goods to meet the requirements of the modern consumer. The areas of this industry which deserve greater attention are:

1. Cotton, ginning and grading, its spinning and weaving on a small scale.
2. Wool, shearing, scouring and grading, combing for further processing.
3. Introduction of high yielding races of silk worm and improvement in silk reeling.
4. Jute ratting and decortication.
5. Flax ratting and scutching on cottage and small scale.
6. Mechanization and improved extraction of hard fibres such as coir, etc.
7. Development of mixed fibres having new properties.

M. Rubber Industry

Demand for natural rubber continues to rise and its production is also increasing in many developing countries. If the processing industry can be developed along modern lines, it can bring better economic returns. Increasing attention is required for:

1. Processing of natural rubber after tapping, pre-processing rubber in various forms, e.g. block rubber, packaging and transport..
2. Manufacture of rubber goods on a cottage and small scale.

N. Tobacco Industry

Production of tobacco in developing countries has been rising. The areas of this industry which deserve attention are:

1. Collection, curing and packaging of tobacco.
2. Manufacture of tobacco products on a cottage and small scale.
3. By-products.

O. Food Machinery Industry

Technological success, quality and standards of the products of agro-industries will depend upon the use of appropriate equipment and machinery to a considerable extent. It is, therefore, essential that a sound programme of design, development and manufacture of suitable machinery for use on cottage, small and medium scale agro-industries should be undertaken on priority. This will help to develop machinery industry and ensure export of good quality products to stand competition on the world market for earning foreign exchange.

Having identified the areas the next step immediately was to raise the competence of developing countries in selection, identification, modification and utilization of these technologies. The developing countries spend less than 2 per cent of the world's expenditure on research, development, technology transfer and training. In fact, Mr. Hulse has given an excellent analysis of this already. It is clear that these resources by themselves are not sufficient to build a suitable mechanism for transfer of technology. While the problem has to be brought to the notice of the Governments of developing countries to increase their allocations for this purpose if they are to achieve technological self-reliance, there is also the need to build inter-country cooperation at regional and sub-regional level. Thus, the developing countries should be able to cooperate in utilizing each others facilities for a common purpose and make their resources go much further. Their problems being similar, the training and research programmes can be developed jointly. Most of the developing countries are located in the tropics. Therefore, their ecological and climatic conditions have a great deal of similarity. Their rural development problems also have a lot in common.

FAO, therefore, has initiated a programme of Industry-Twinning under which technology can be transferred from one industry to another and also from one institution to another or to industry. Another step taken simultaneously by the Organization is to create a mechanism or an infrastructure for this purpose. It has been proposed that the food and agricultural industries development problems should be clearly identified, government policies on the subject and the resources allocated by them understood and then a programme of cooperation should be built. Such a programme could be a network of institutions at a regional or a sub-regional level. The coordination centre could be allocated at one of the national institutions. This would minimize expenditure in terms of cost, facilities and personnel. It has already been proposed that a Technology Transfer Centre for food and agricultural products conservation and processing industries for Asia should be located at the Central Food Technological Research Institute, Mysore where FAO has been running an International Training Centre for the last 9 years. Mr. Hulse, who is present here, made a valuable contribution as Chairman of the Canada-Mysore Project for building the Centre. 16 countries of Asia are already participating in it. Studies are already in progress to establish similar centres for Africa, Near East and Latin America. In addition, FAO is preparing a list of appropriate technologies by collecting the information from developing and advanced countries as well as from institutions which have developed such processes and have ensured commercial success. I take this opportunity to appeal to members of the Panel and the participants to provide such technologies to FAO, so that they can be utilized for building, preferably indigenously owned, food and agricultural products conservation and processing industries. The Assessments of the World Food Situation prepared for

the World Food Conference already points out that food losses are between 20 to 40 per cent. These losses are not only quantitative but qualitative. Their reduction through the development of conservation and processing industries is fundamental to achieving self-reliance, raising the standards of nutrition and improve productivity of the population.

Development was conceived at one time as an extension of the scientific, technological and management know-how available from advanced countries to developing countries in most cases, are not appropriate to the requirements of developing countries, not are the old obsolete technologies which the advanced countries were using 50-100 years ago suitable. A time has come where alternative, appropriate, modern, science based technologies have to be developed and utilized. Such technologies must be capital saving, labour intensive, poverty reducing, capable of further growth and development and should be able to manufacture products of high quality for internal consumption and export. I recognize the problem of combining all these factors into one, but with a systematic effort it should not be impossible to develop technologies which can meet most of these requirements. I would, therefore, take this opportunity to appeal to my colleagues, specially from the developing countries to increase cooperation between themselves and to my colleagues from advanced countries for providing support in the form of specialized training and in providing resources for applied research to build such technologies in the developing world. Science is universal, but technology as a distinct social, economic and cultural orientation. Yet it must be recognized that economic progress is directly proportional to the use of science and technology for harnessing natural resources for the benefit of mankind. This becomes clear when one has a look at the map of the world. Although the developing countries represent over 70 per cent of the world population they account for less than 40 per cent of food production and less than 20 per cent of the world GDP. I feel that a food technologist can play a vital role in changing this picture and improve the quality of life for mankind.

Now Dr. Barber, of behalf of Professor Primo, is going to summarize the functions of research and development institutions in this area of technology transfer.

Salvador Barber on behalf of Eduardo Primo

Mr. Chairman, ladies and gentlemen, first of all I should like to apologize for Mr. PRIMO not being able to come here at this moment, as you can realize the president of the Congress many times has some unforseeable appointments. In this place I will try to read the paper and I will try to answer any question concerned with it. My language is Spanish.

Uno de los factores que definen el desarrollo económico y social de los pueblos es el volumen y la calidad de su investigación científica y técnica. La investigación promueve el desarrollo tecnológico, que es base del desarrollo económico. En muchos programas de investigación, los resultados tienen carácter universal y pueden aprovecharse en todos los países si estos disponen de la información necesaria y de los técnicos y de las condiciones adecuadas para aplicarlos en su área local. Pero todos los pueblos tienen problemas tecnológicos propios que frenan su desarrollo y que exigen una investigación específica para resolverlos y obtener las soluciones concretas que precisan su peculiar índole y circunstancias.

Dentro de la tecnología de alimentos, esta peculiaridad y diferenciación será de mayor grado que en otras disciplinas, debido a sus diferentes materias primas, costumbres, gustos, dietas y situación económica. En los países que están en vías de desarrollo, la detección de los auténticos y más urgentes problemas de esta clase es una necesidad primordial. Sobre esta prospección debe basarse la organización de un potencial investigador adecuado para encontrar soluciones y desarrollar la tecnología necesaria. Hasta este punto, la acción exigida cae dentro del campo de las actividades de investigación y desarrollo, en la que son competentes los centros de investigación en ciencia y tecno-

logía de alimentos. El aprovechamiento posterior del conocimiento tecnológico para alcanzar la producción innovada, entra en una esfera política y financiera, en la que los organismos de investigación, sólo pueden aportar su información y su asesoramiento.

La producción, en los países que están en vías de desarrollo, de alimentos nuevos o más convenientes, el aprovechamiento de nuevos recursos para nutrición de la población, para la implantación de procesos nuevos o perfeccionados, exige la aportación de bienes de producción y una acción de promoción y educación, con las implicaciones políticas y económicas que todo ello supone. Finalmente, el mantenimiento de una producción estable y autóctona, exige la formación de técnicos.

La ayuda a los países en desarrollo puede realizarse en cualquiera de las fases del proceso generador de la producción de bienes de consumo. Y, evidentemente, también por la donación de éstos, que es la forma más utilizada de asistencia. Sin embargo, conviene puntualizar que ésta será tanto o más eficaz, adecuada y de efectos más permanentes, cuanto más se aproxime a las fases iniciales de dicho proceso generador, siempre que pueda asegurarse el cumplimiento de las siguientes hasta alcanzar la producción innovada. Supuesta una voluntad colectiva de ayuda de orden internacional, estas actividades han de tener una secuencia definida y programada, como una cadena de acontecimientos, que deben atribuirse a organismos de distinto carácter. En este sentido, sería de la competencia de los organismos de investigación la realización de las primeras fases, es decir, detección de los problemas que frenen el desarrollo y de las posibilidades no explotadas, y organización y realización de la labor de investigación y desarrollo necesaria para obtener la tecnología adecuada para ellos.

La ayuda que se preste en la investigación es de incalculable y multiplicativo valor, porque ataca al problema en su propia raiz: una causa radical del subdesarrollo es la falta de investigación. En el caso de la ciencia y la tecnología de alimentos, todo esto tiene mayor gravedad y trascendencia. Esta forma de ayuda primaria podría alcanzarse mediante un concierto internacional, por el cual los grandes centros de investigación de los países avanzados asumieran las tareas de resolver un problema de un país en vías de desarrollo como una parte de su programa de trabajo. Puesto que los grandes centros de investigación son en la mayor parte estatales, ésto exigiría el consenso previo de los gobiernos.

Junto a esta labor de prospección y de investigación y desarrollo, se debe organizar la formación de técnicos y de investigadores que desarrollen la semilla inicial. Es evidente que la realización de un programa de investigación centrado en problemas regionales, exige el contacto con las circunstancias locales, lo cual tiene importantes inconvenientes cuando están situadas a grandes distancias del centro investigador. No obstante, una buena planificación del trabajo y el contacto siempre posible y necesario con personas y organismos del país, pueden allanar las dificultades que siempre son vencidas si existe auténtica voluntad de ayuda. Como un ejemplo reciente de ayuda exterior para la prospección de problemas no resueltos de países en vías de desarrollo, puede citarse el trabajo realizado por la Commission on International Relations of National Academy of Sciences, National Research Council, de los Estados Unidos. Este trabajo ha dado lugar a una publicación titulada "Food Science in developing countries: a selection of unsolved problems", que es de gran interés como base para una acción conjunta como la que se propone. Pero los problemas más graves comienzan cuando el problema tecnológico está resuelto.

Supongamos, como un ejemplo, que se ha desarrollado un proceso para aprovechar un subproducto depreciado y obtener un alimento proteico que permita mejorar la dieta de la población en un país en desarrollo. Incluso se da fin al trabajo, con el proyecto de las industrias transformadoras y su estudio económico, que resulta favorable. La instalación y puesta en marcha de estas industrias en estos países, su mantenimiento, la distribución y uso de los productos, la educación de la población para su consumo, etc., implican problemas muy graves de orden financiero y político, que sólo las más altas organizaciones de carácter internacional pueden resolver. Pero aquí, y de

un modo eficaz, los organismos de investigación científica y tecnológica pueden prestar una ayuda indispensable mediante su información y consejo. El potencial de información científica y tecnológica que se acumula en los centros de investigación, tiene un valor incalculable para tomar en nuestro tiempo cualquier decisión política o económica.

Esto nos lleva a considerar las escasas relaciones que hoy existen entre las grandes organizaciones internacionales de carácter político o económico y las organizaciones investigadoras de los distintos países. Es cierto que existen y se constituyen numerosas comisiones en las que participan científicos expertos, que pertenecen en muchos casos a centros investigadores. Pero es indudable que el centro tiene un potencial de información superior al de cualquiera de sus científicos y que en sus programas de trabajo puede incluir la colaboración con organismos internacionales para el estudio de problemas tecnológicos, de reglamentaciones, de métodos analíticos unificados, etc. Esto sería muy beneficioso, tanto para la selección de los problemas como para un mejor aprovechamiento de las soluciones y de los resultados. Por otra parte, las oficinas y gabinetes científicos y técnicos que existen en dichos organismos internacionales y que están constituidas por muy destacados especialistas de la ciencia y de la tecnología, pueden ser los mejores órganos de enlace entre aquéllos y los centros de investigación. Este simposio, en el que están reunidos prestigiosos dirigentes de organizaciones internacionales con investigadores de la ciencia y tecnología de alimentos, puede ser una buena ocasión para meditar sobre la forma de mejorar estas relaciones.

H.A.B. Parpia

I am sure you will agree that the points made by Professor PRIMO, as presented by DR. BARBER, are of immense value especially with regard to identification of the problems and building of the professional institutional mechanism.

Now, I would like to invite DR. ACHAYA to present a brief report on a Workshop that was organized here by the League of International Food Education. The subject was "How can food technologists in developing countries focus their effort for responding effectively to major in-country food and nutrition problems? ".

K.T. Achaya

The Workshop was attented by about a dozen countries from Asia and the Far East, South and Central America. The recommendations have been roughly drawn up, and what I have to say is really a kind of draft impression rather than full report.

The Workshop identified four major areas which required attention if food sciences and technology were to be effectively applied to better feeding of people from developing countries. It sought to give, in these areas, a practical and workable tone to its recommendations, I will very briefly mention them now.

The first major area was "improved communication in food science and technology" We all are aware of how knowledge in food science and technology is growing at an extraordinary rate and, therefore, mechanisms are required for its effective dissemination. The Workshop felt that to avoid duplicating efforts, waste of time and money, wider use should be made of the Food Science and Technology Abstracts. It was suggested that regional documentation centres, at suitable locations, should select and distribute from this abstract, information in the ways most suitable for use by local industry, government or institutions. These regional documentation centres could establish not only strong links among themselves but also provide a feed-back system on reverse information flow to Food Science and Technology Abstracts. Such centres could also play a useful role in educating people about facts on food science and technology which affects their habits and daily life.

To attune food science education more closely to country needs was the second area identified by the Workshop. It was suggested that more country institutions must

be set up to impart training carefully geared to the actual needs. Multilateral and bilateral help could be sought for the creation of such in-country institutions. Appropriate curricula both theoretical and practical should be developed by local and regional groups for such training organizations. Suitable text books and related literature should be envolved as a defined objective. It was suggested that the students could most appropriately spend their senior year with a local industry.

The third area was to take measures to improve the status of the existing in-country food science and technology institutions. Some of the steps suggested were to establish close links with the local government and industry, develop strong extension services, set up mobile demonstration units and organize short courses. Concrete steps in these directions could also be taken by outside multilateral or bilateral agencies. Some of these could be to prepare lists of food science and technology institutions in each country giving information on their areas of work, personnel facilities, resources and so forth; refer enquiries received from the countries to these institutes in order to build their own confidence for self help. This in turn would imply that these in-country institutions should be helped to set up an effective problem-solving liaison system.

The fourth area to which attention was considered necessary was in improving the in-country research and development infrastructure. Food science and technology can only be as effective as the total system into which it is integrated. Perhaps a model of what constitutes a suitable infrastructure could be developed through surveys and questionnaires. In-country institutions, in turn, must give high priority to studies aimed at prevention of food losses and to utilization of food and agricultural by-products. Some of these areas were briefly defined, such as devising measures against field losses and damage caused by insect and rodents, devising appropriate storage facilities, working out methods for agricultural by-products utilization, devising practical ways of reducing losses in the food processing, helping governments to lay down appropriate standards and specifications for foods.

In short the Workshop recommended that maximun publicity must be given to the need for solution of the problem in the publications of the UN system, the LIFE* newsletter and professional food science and technology journals.

This was the gist of the recommendations made by the Madrid LIFE Workshop.

H.A.B. Parpia

Thank you very much DR. ACHAYA for giving us this brief report. Now I want to invite the discussants from the audience to make their comments and suggestions. May I begin by inviting DR. PHILLIP SPENSLEY, Director of the Tropical Products Institute, London, to comment.

Philip C. Spensley

The previous speakers have well covered the ground concerning this matter of technology transfer. For the purpose of the discussion, however, I would like to put one thought forward.

The transfer-of-technology chain with which we are concerned has three main components, (1) the need for a particular technology in a developing country (2) the technological institutes in the country which should be concerned with providing this and (3) the help that is required from outside the country in acquiring the needed technological knowledge and experience.

The links between (2) and (3) are often discussed and have been quite extensively explored and developed. For instance, my own institute, the Tropical Products Institute in London, has many co-operative links with institutes in developing countries through

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"League for International Food Education"

which technology transfer takes place in various ways.

The even more vital link, however, is that between 1 & 2; and this in some countries may almost be described as "the missing link".

I have in the course of my contacts with Scientific and Technological Institutes in developing countries found that a number of them pride themselves on their independence – their autonomy; but, in my view, this is not an attribute that serves the institutes or their countries at all well. The institutes may enjoy the freedom to choose their own projects but if these are not integrated with national and industrial developmen plans, the institutes may become "ivory towers" and then be treated as such to their detriment.

The importance attached to autonomy by these particular institutes seems perhaps to be a hang-over from, and to be confused with, the kind of independence and academic freedom which were fashionable even for supposedly applied scientific establishments in countries such as the United Kingdom up until a few years ago. Now-a-days, however, it is becoming accepted that government-sponsored applied scientific activities should be controlled by the bodies with executive responsibility for the subject areas where the problems arise and according to priorities determined in the national interest.

This represents some loss of autonomy for the Scientific organisations involved, but I suggest that institutes in developing countries should accept this in return for being placed, as they should be, in a prominent position in the national development scheme of things, where they will be fully utilized and given a greater involvement in the decision making process. In this way the scientists and technologists will be steered into the more important activities from the national point of view and at the same time will increase their influence and standing.

H.A.B. Parpia

I would like to comment on DR. SPENSLEY's statement.

There is a difference between autonomy for action and academic freedom to work on hobbies. The demand of institutions for autonomy, is to permit direct dealing with industry and several different departments of the central and state government to deal with their problems, rather than to become a part of a rigid bureaucratic machinery. This is where autonomy has a real meaning. I agree with DR. SPENSLEY's view, that institutes should be involved in the development planning. To achieve this they should have sufficient freedom to express their views in the national interest and be a party to the final decisions for action.

Academic freedom can go too far where paper publishing can become the main objective. This is something which can be highly questionable for a technological research organization, which has to solve specific problems for fulfilling their objectives. I think the subject is worth discussing further, keeping in view that the main objective of these institutes is to deliver the goods and to establish their credibility.

The next discussant is DR. PÉREZ VILLASEÑOR, Professor of Food Technology from the Metropolitan University of Mexico City.

Javier Pérez Villaseñor

Although previous speakers have mentioned the topic of training of personnel I shall go a little further into it and share with you some of our experience in building of integrated competence in food science and technology in Mexico. Due to the short notice given to me to speak here, the views I express may be rather incomplete in some respects. Therefore I ask for your forgiveness.

Since we are talking about food science and technology I would like to start by setting up at least three main objectives for developing a programme of science and technology for a developing country and specifically for Mexico. Such a programme

should be able to identify the training needs with a view to solve the problems. I think this should be the first objective. The second one should be that the solution of these problems in science and technology should encorage the economic development of the country, state or region. The third objective of any such programme should be to develop new technologies through scientific research and to ensure their utilization.

Having set up these minimum objectives, in relation to the subject of training personnel, I would like to quote a Chinese proberb which says "Give a fish to your hungry bother and he will eat today; teach him how to fish and he will eat forever"; This is a very sound thought and stresses the need for achieving that self-sufficiency through training personnel. Such an effort should have priority in developing any programme on transfer of technology. In Mexico we are trying to build multi-disciplinary staff competence in food science and technology. Teaching and research staff are being selected initially to build an integrated programme of work, with due attention being given to leadership and management capacity. After this group is assembled, we shall be faced with the problem of making a decision whether to send them for training abroad or to train them in the country. Since in most of the underdeveloped countries, including Mexico, good advanced training programmes in food science and technology do not exist, the decision is clear that people should be sent abroad for training. One would like to send them to the most advanced universities which provide the best curricula. We have found that between two and five years are usually required for them to become proficient in the new technologies, but unfortunately staying abroad so long results in their losing contact with the problems of their own country. Once they finish their training in these advanced countries and return home, they are faced with an entirely different atmosphere from the one under which they learned to work. They inmediately find that they cannot get the high salaries. They lack understanding of the problems to be solved and the working conditions are different from what they have got used to. They become discouraged and leave their countries, contributing to the brain drain which saps the resources of these countries.

I believe, along with many people, that in sending people to be trained abroad, one should select a country which has similar conditions and similar problems, and develop within the country itself an institution of high standard with proven competence in improving the conditions of the country. I am referring to places like the Central Food Technological Research Institute, Mysore, India. India being a country with similar problems to the ones we have in Mexico, Nigeria or some other country.

I shall refer now to the curricula offered at the different institutions throughout the world in advanced countries in the field of food science and technology. The courses are extremely well taught and supported by laboratory and pilot plant work, but one must be aware that these courses may not provide the exact type of knowledge required by a foreign student when he returns to his country. The curricula are designed for preparing the people needed by the advanced countries themselves. In several cases they leave out, in the preparation of foreign students, courses in post-harvest conservation of food grain, physiology of tropical fruits, traditional packaging and conservation of fruits and vegetables in their fresh state, chemistry and technology of cereals and a few other courses which can be of great benefit to these students once they return to their country.

I shall refer now to the preparation of a curricula in a developing country. These curricula, both for graduates and undergraduates, should not be a copy of what students learn abroad. The curricula should take into consideration the problems and needs of the country and its natural resources. Once these have been identified priorities must be laid down and courses designed to ensure that on completion of training the student can be of value to his employees. Once this is achieved we are half way to creating a strong base for the selection and transfer of food technology. Yet the task is quite incomplete

because most of these countries do not have access to the necessary information published in specialized books and journals, as they cannot afford to buy such expensive publications. Efforts must be made to set up an information and documentation centre to collect and provide latest information at the country and regional level.

Having trained personnel, having built facilities and the information centre, what next is to be done about the transfer of food science and technology to the country from various sources? The transfer of technology should be done from the instituttions to the industry, to the farmer, to the government and to other educational institutions and from industry to industry. The farmer should be included because he needs assitance of an extension service in regard to pesticide use, pest control, infestation, storage improvement etc., in order to supply the required quality of raw material. He lacks this knowledge and therefore the losses, both pre-harvest and post-harvest, are heavy. In Mexico alone losses of agricultural products run as high as 20 thousand million pesos. The Government needs assistance in several ways; first of all the Government should set up food regulations, and quality standards should be brought up to date. The Government should be advised on what should be done, for instance, in building food security specially in times where there is a drop in production and the reserves have come down to a very low point. Obviously, this group of technologists should also provide the government with the necessary advice to select technologies from abroad for developing industries. Much has been said about technology transfer from the institutions to the industry; therefore I will not go into further detail on this point.

In summary, I would like to suggest that transfer of technology should be clearly defined because many people, do not have a clear idea of this. And in doing so, international organizations might consider the organization of regional seminars or courses on technology transfer, and also for setting up regional centres for selection and transfer of technologies so that the countries can become self-sufficient in the training of the people they need and improve their competence to assist other countries in reorientating food research to produce the desired impact on their economies.

With this I shall close my comments.

H.A.B. Parpia

Thank you very much DR. PÉREZ VILLASEÑOR. We certainly appreciate your suggestions regarding the training needs not only for students but also for the institution builder and the manager of good industries in developing countries, the provision of facilities for work and the need for the establishment of institutions for the transfer of technology.

I shall now call on the next speaker, DR. AMARA BHUMIRATANA, who has been very much involved in Technology Development and Transfer in his country, Thailand.

COOPERATION BETWEEN RESEARCH CENTERS AND INTERNATIONAL ORGANIZATION FOR TRANSFER OF TECHNOLOGY TO DEVELOP FOOD INDUSTRY*

Amara Bhumiratana

Institute of Food Research & Product Development. Bangkok, Thailand

Mr. Chairman, Ladies and Gentlemen, I am very honoured to be invited to give comments here this afternoon. I feel very honoured since I am a member of a less developed country, Thailand.

What the speakers (DR. STEWART, DR. ACHAYA, DR. HULSE, DR. GORGATTI-NETTO, and Mr. Chairman) said this morning were undeniable. However, as a member of a less developed country, I would like to make an observation that the speakers were looking

*Text provided by the author.

at the problems from the points of view of members of well developed countries. They were looking at weak points seen in less developed countries and tried to improve the conditions in those countries through "technology transfer".

My first comment is on the paper given by DR. SPENSLEY about technology transfer with regards to research and development which will result in improving industries in less developed countries. Since I am working at the Institute for Food Product Research and Development, I would like to relate my experience to the problem of technology transfer from a well developed country to a less developed country.

We must examine the problem in terms of the *needs* of the people. In Thailand, people eat to fill up the stomach with grain products (rice) and not with protein food. Therefore, at the Institute we studied "food and nutritious food" and the eating habit of the people by conducting a project on protein food development and protein food promotion. The project was supported by the USAID and was conducted by Thai staff. The Australian government supported a project on baby food to try to improve the quality of baby food intake. Thai babies have been depending on sweetened condensed milk which have very little nutritious value. In Thailand children ages 0-5 need supplemented protein to the normal food intake to become physically and mentally strong. The facts stated here show that we are learning about conditions and needs of the people in our country. And, for the benefit of our people we are trying to promote baby food and high protein low cost food programs.

Another project being conducted to serve the needs of the people is the algae project. The project has been supported by the West German Government. A preliminary survey of the project shows that a group of Thai people consume fresh water plants which look like algae. The West German Government and the Institute have been working together on this project. At the present the project is at research and development stages and have not reached the stage where technology is being transferred to industries. One problem which retards the technology transfer stage is the fact that the products will be consumed by low-income people and the price of the product must be low. The industrial investors are not willing to invest for small marginal profit until they are sure of the market acceptability. Therefore, for the benefit of the low income people the government must step in. The Institute is now producing high protein products requested by the Public Health Department. The government has set up a budget to buy high protein food for the target group.

What I have informed you are examples of the cooperations and supports of the well developed countries to less developed countries at the institutional levels. In addition, the Institute has set up a short course program for the need of the factories by sending mobile units to the factories regularly. The information given are on technology, preservation, retort operation, hygienic practice, etc. This is an example of an attemp to extend the aids given by well developed countries to the industries in less developed countries and trying to utilize local products and request only what are needed.

DR. ACHAYA mentioned that the well developed countries do not want to build new empire and the less developed countries do not need very modern equipment. With regard to this point I would like to state that the problems arose because members of the less developed countries believe the experts blindly. The experts in the past had suggested and requested new institution and new equipment without trying to utilize the existing commodities. The equipments given by well developed countries are transferred directly to the less developed countries without adaptation. In some cases, the technology transfer of mining equipment and technology, for example, have worked well. But with regards to food technology, technology cannot be transferred blindly. The experts come to introduce new ideas and technologies and leave after their terms are over, and Thai technicians have to take care of the rest of the project. Therefore, local people who work with experts must try to work cautiously with experts and accept what is applicable and adapt what is not directly applicable.

At present in Thailand the National Food Science and Technology Center has been established at the Institute where I am now the director. The center will function as the coordinating center of all food personnels in the country. It is where food personnels will meet regularly to exchange ideas and information. The first project is to organize a who is who directory which will include information on individuals, projects, types of support of the projects, and results of the projects.

Finally I would like to emphasize that technology transfer between members of the region and sub-region should be encouraged. At the regional and sub-regional levels where developments are at the same stages, where problems are similar, where aims are not very different, and information and ideas may be exchanged, is where the well–developed countries may give aids to the countries in the region or sub-region collectively. The aid will then be spread furthur to national, and sub-national levels. This will be an incident where technology is transferred from well developed countries to less developed countries and among less developed countries themselves. The problems of adoption without adaptation will be reduced since technology will not be transferred directly from well developed countries to less developed countries but technology will be transferred and evaluated at the regional and sub-regional levels.

At the Institute where I work, many groups of Laotian have been trained on protein food program. The programs were supported by the USAID. There have been 5 groups coming from Laos for the period of 3 months each. A group from Nepal had also come to be trained on quality control. A regional quality control training group had included people from Korea and Malaysia. These are examples of technology transfer at the regional levels.

Another case of technology transfer which is important in less developed countries is the case of post harvesting problem. This is the problem recognized by the Thai government. Both the Canadian and Australian governments are now interested in bringing this problem to the attention of members of the national centers. This is a big problem concerning different groups of people from the time food leave the producers to the time food reaches the consumers' mouths. Government offices involved in the process includes:

The Ministry of Interior – the labor force problems
The Ministry of Agriculture – problems on farm technologies, agricultural extension units, etc.
The Ministry of Industry – industrial management problems
The Ministry of Communications – transportation problems
The Ministry of Commerce – marketing problems

Therefore, it is seen that in trying to serve the need of the people in term of food supply, many groups of individuals are involved. In trying to help, well developed countries in the past tend to send one expert such as an agriculturalist to help when in fact a team of experts is needed. This is one weak point which have been detected. It is hoped that the formation of national centers will help improve the conditions in the less developed countries and avoid having problems similar to problems seen in the past.

The report of L.I.F.E. which supports the development of regional and sub-regional food science and technology group need to be fully supported here for the benefit of less developed countries. This meeting should support that proposal and try to bring about technology transfer at the same time. Cooperations between groups at the national levels will minimize cost and improve quality simultaneously.

I would like to emphasize again that each country knows her problems best. She knows what is needed most. In some countries, food is needed and in other countries, nutritious food is needed. We cannot wait for the rise in income of the people alone. Thus, in the fourth 5-year plan, nutritious food will be given to people under the economic and social welfare programs. This is not the work of one institution but dif-

ferent institutions must work together to solve the problem of the need of the people. We must work with existing equipment as best as we could. We must try to help ourselves first. With additional aid from well developed countries, the eating habits and health of the people will be improved.

I am very honoured for the opportunity to give my comments and I am sorry to have to state some unpleasant facts, especially the one relating to the experts.

Tai-Wan Kwon

I might mention the problems we have in Korea on transfer of food technology. I am from the Korean Institute of Science and Technology or as we call it KIST. The purpose in setting up this Institute was to help the Korean Food Industry by contracting research and development work which the industry cannot carry out by themselves and we have been able to make significant progress. This Institute does not belong to the Government and therefore, burocracy is avoided.

H.A.B. Parpia

Thank you very much DR. KWON. I have visited your institute in 1970. I think you comment has special value because of the pattern of problem identification which you have been able to develop and which is now being increasingly adopted, where the potential economic returns of the results to be produced has to be determined first. Your effort can be of special importance to small industries who are not in a position to undertake their own research who come to you for assistance to formulate projects of their interest based on their problems of economic value.

I now request Professor M. JUL, who has such a lot of experience from which we can learn. He has been a critic of hobby oriented research. This approach is essential if any realistic results are to be achieved.

Mogens Jul

The Chairman asked me to say something, and as a matter of fact suggested that I be the Devil's advocate.

I would like first to stress my complete agreement with the Chairman. It seems that we discuss this subject as if industrial development, and here we talk about food development, is a question of good technology, good ideas, etc. If you give good sound advice on new type of food technology, a new food industry is expected to spring up in a developing country. Let us agree, that this is far from being the case, and in fact many disappointments can be traced back to this oversimplification. The establishment and viability of a food industry requires, besides technological know-how, management talent, adequate finance, marketing function, suitable personnel both at the executive and the operational level, etc. Secondly, however, some of this discussion has had me quite worried. It seems that we talk about research laboratories, training of university graduates, as this were the only prerequisite for having a suitable technological process accepted in an industry. I only seriously question if it is more science we need. Do we not already have mountains of scientific work which is not being used. Is it not so that anyone adding to the mountain of scientific, or for that matter technological, literature, adds to the mountain of literature which operatives do not have time to read.

I submit to you, Mr. Chairman, that we are close to what I term the unscientific use of science. It is unscientific to proceed as if the world needed more science, if it is not using what has already been produced. It is also unscientific to go on producing science without looking after its utilization and implementation when we talk about technological matters.

What I say has no reflection on pure science, the prerogative of the universities and other institutes of higher learning. It does however, reflect on such technical ef-

forts which purports to be carried out for the benefit of industry. Where-ever that is the case I feel that the activity, call it science, research, or whatever you like, must be held accountable. If a person obtains funds for some assistance to industry, I reserve the right to come back to him in 6 months or maybe 1 or 2 years time and ask just what did you get out of it. Is it being used by industry, and my only criterion for his success or failure is whether his efforts have found application.

I would like to remind our friends from the low-income countries that they can learn little in this respect from the so-called developed ones. I am may even add, that especially the graduates of our universities may not be the right persons always to help them. There is one thing that always struck me as rare, whenever higher education is discussed, it is stated that it should also be combined with research. I always wonder why it could not be connected with development. The fact is that it is not. Keep that in mind, because that may suggest to what degree university trained people have experience in development.

I myself had an experience which may demonstrate my point. I was then with FAO Fisheries Division. I had a request for a specialist in long-line tuna fishing. An expert of that kind was quite hard to find. The art is mainly developed in Japan and at that time few were available, who spoke English and had proficiency in long-line tuna fishing. We found one, and proposed to the country that he be accepted. The country cabled back and asked for his academic qualifications. I answered with what was probably the most undiplomatic cable I have ever sent, I simply stated: he has none, he can fish. Anyway, he was accepted.

Recently I visited some research institutes in low-income countries. Actually, it seemed to me that they were closer to their industry than those in the industrialized countries often are. At least in these cases they could well serve as models for us. Conversely, I recently discussed the problem with a person from a technological research institute in a industrialized country. He lamented the fact that industry was not receptive to their work and did not use the results. I said, you should have some consultants to aid you in the technology transfer and contact with industry. He said that he have no funds for them. I said, how many professionals are you. The answer was about 50 and I then asked why not 10 of them were consultants. He then actually said that they could not do that because this was not within their terms of reference. I am afraid that I was rather rude when I simply suggested that they go and change their terms of reference.

In this respect I liked what has been mentioned once by our Chairman. In university research the slogan at least in USA has long been: Publish or perish. In industrial research the slogan might rather be publish and perish. If you are close to industry you do not need to publish, you talk to them and you do not have time to write because they are too eager to use your know-how.

The problems, as I outline them, are probably especially true for the food industry which by and large is a primitive industry at least in the industrialized world. We have had some cases where industry groups in Denmark have asked for some sort of research facility. I have suggested that they get one consultant with an amble budget for travel, telephone, and the most important literature, and that he be kept far away from a laboratory bench. It actually has worked.

In my own case I have recently taken over being in charge of an industrial food research institute. It has in its name a research institute, but I have firmly stated that our aim is to avoid doing research, and we tell that to anyone who seeks employment with us. We have travelled all over the globe to find out what is new in that particular industry, we keep abreast of developments whereever we can find them and we have made it our objective to know the Danish industry inside out. As I have stated our aim is to become a member of the industry family, they should feel us as their equal, and just as important as any other function they have. I think that we can say we

have succeeded in that we are overwhelmed with requests for assistance, and have no budgetary difficulties.

H.A.B. Parpia

Fortunately research, in general, is becoming more realistic as a result of the interaction of scientists with social problems and there is need for more conscious steps in this direction. We all should think more along the lines suggested by Professor JUL. I hope there will be more discussion on this subjects.

The next comment is from DR. REYNA of Argentina.

Rodolfo Reyna

Thank you very much DR. PARPIA for giving me the opportunity to speak. As DR. BARBER said this morning, my language also is Spanish and so I apologize because I am going to use this language. También quiero expresar mis sinceras disculpas porque la exposición que voy a realizar no siga una línea demasiado ortodoxa, pero lamentablemente no he conocido con la antelación necesaria los temas de esta mesa redonda.

He venido escuchando con vivo interés las opiniones presentadas hasta ahora y he sentido, junto con algunos colegas argentinos, la necesidad de exponer algunos comentarios que éstos me han sugerido. Mi solicitud de intervención fue formulada al DR. PARPIA con aterioridad a su actuación en la que tan magistralmente expusiera la idea sobre un banco de transferencia de tecnología , idea que puede constituir probablemente la respuesta a mis inquietudes personales que representan el sentir del Instituto al que pertenezco, y seguramente también el de muchos colegas de mi país. Cuando esta mañana escuchaba al DR. GORGATTI NETTO renacían en mí muchos de los problemas que Argentina viviera cuando decidimos planificar nuestras necesidades de investigación y desarrollo en tecnología de alimentos. Al igual que Brasil, experimentamos la dificultad en planificar las líneas de investigación y algunos de los presentes han sido testigos de ello porque fueron consultados por nosotros, cuando hace ya unos años, iniciamos nuestras actividades en tecnología de alimentos. Conscientes de la responsabilidad que significaba para los tecnólogos de alimentos de un país como Argentina, de enormes recursos básicos, la orientación de la industria de este sector, planteamos la necesidad de desarrollar líneas para mejorar al nivel institucional los alimentos de consumo, mejorar la calidad de los mismos, brindar nuevas perspectivas y aumentar el valor de nuestros productos de exportación. Todo ello sobre la base de adecuar nuestro desarrollo a nuestra propia personalidad, a nuestra economía de escala y por supuesto, a nuestras propias limitaciones.

Nuestro país ha recibido, en función de estos programas y de sus necesidades, un apoyo eficaz por parte de organismos internacionales. La colaboración de la FAO con nuestro Instituto Nacional de Tecnología Agropecuaria (INTA) ha resultado decisiva en la potenciación de la producción de materias primas para alimentación y la ayuda de la UNIDO, prestada en forma de envío de expertos, ha sido destacada en los campos específicos para los que habían sido requeridos. Sin embargo, el equilibrio de nuestras posibilidades, el interés de nuestras industrias y, además, el interés internacional en contribuir a las necesidades nutritivas mundiales, nos han hecho comprender que también Argentina puede desempeñar, en alguna medida, un papel eficaz en la lucha contra el hambre. En este sentido creemos que, dada la etapa de desarrollo en que nos encontramos, necesitamos un nuevo tipo de colaboración: aquella que nos brinde la posibilidad de llegar con nuestros productos transformados a partir de nuestras propias materias primas a los lugares donde escasean o faltan alimentos. Nosotros producimos trigo de muy buena calidad y en mucha cantidad, nuestras harinas gozan de una favorable aceptación en el mercado internacional y, sin embargo, no hemos abordado la posibilidad de estudiar el enriquecimiento de estas harinas. Tenemos una industrial alimentaria en expansión, una

sólida industria textil y una industria papelera seriamente apoyada por el gobierno. Creemos que estos tres sectores industriales necesitan un buen apoyo tecnológico para el empleo de almidones modificados ya que ello permitirá la transferencia, a niveles económicamente aceptables, de nuestros excedentes de harinas fortificadas. Esta política es interesante para los países hispanoamericanos ya que se les brinda la posibilidad de desarrollar sus propias perspectivas con mezclas de harinas locales.

El caso de nuestras industrias pesqueras y cárnica presenta también una seductora alternativa que no hemos explotado. Teniendo en cuenta que existen en Hispanoamérica Institutos capaces de transferir tecnología, y de hecho lo están haciendo, creemos que, de alguna forma, en combinación con ellos se podría coordinar la transferencia de la tecnología más idónea para nuestros productos, necesidades y posibilidades. Hasta ahora, sin embargo, el apoyo desinteresado y valioso de los organismos internacionales tan solo se ha limitado a la solución de problemas muy concretos a nivel de tecnología aplicada y ello siempre motivado por deseo expreso nuestro. Por ello creo en la necesidad de un organismo que nos enseñe las posibilidades de poder contribuir coherentemente con nuestros propios recursos a la urgente necesidad de alimentos en el mundo. Creo que este era el deseo del DR. PARPIA cuando esta mañana aludía a la necesidad de crear un banco de transferencia de tecnología y en este sentido puede contar con nuestra incondicional ayuda ya que es el sentir general de los tecnólogos argentinos. Gracias por su atención.

H.A.B. Parpia

I would like to stress now one point from PROF. PRIMO's paper: the need for identification of the problem and mechanism for the transfer. Any suggestion on this will really be of great value.

Our next speaker is DR. HOWAT from Nigeria.

G. R. Howatt

I wish to make these brief points

1. To underline the need for awareness of the sociological and economic background in the transfer and application of food technology. The infraestructure is often very weak, especially for village-type industries. Goods and services taken for granted in developed countries may not be available. Piped water is one of these.

A few months ago I spoke to a woman preparing gari – a staple carbohydrate food. She was washing peeled cassava tuber in stream water which was obviously contamined with oil from a work-shop upstream. I asked why she was driving this work in poor quality water. She said, correctly, "Where else can I go? ".

2. The training of postgraduate students overseas has already been commented on adversely and –in my view– correctly. It may interest this group to know that the University of Ife is already following the policy of having our graduate students registering for doctorate degrees in the United Kingdom and doing most of the field work in Nigeria. This is in line with our policy in the Department of doing research on existing foods for which a known demand already exists. We try to find out if it can be prepared in a different (and better) way, if the hygienic conditions of manufacture can be improved, if its nutritive value can be improved, and if its "shelf-life" can be extended.

3. It was suggested that educational institutes should endeavour to cooperate with the food industry to their mutual benefit. This is an excellent proposal but it is not easy to do. Often industrialists are sceptical of the potential contribution of Universities. The latter have to show that they can make an effective contribution. Although our Department is still very young we have in fact already attempted to do this and at our students' Food Science week we had a useful University/Industry seminar. Another one has already been arranged.

Joseph H. Hulse

Thank you very much DR. HOWAT. I was delighted to hear that this idea of studying the theoretical aspects of the problem in the developed country and the practical problems in the developing countries is actually in effect. This is most encouraging. May I now call upon Dr. Debling from Grimsby College of Technology.

Graham Debling

I did not go for education with the idea of wasting my time doing research. I do not mean to insult the people present here, forgive me. I went into industry.

Much has been said and done about the transfer of basic technology. To this end, we, at Grimsby, run a twelve months' course on fish management for the fish industry from developing countries. This is a very broad course, only 20 per cent of the work relates to fish handling. Many of the participants have been people working in the field and in government laboratories and very few of them have any appreciation of food technology. We do our best to rectify this by emphasizing in the courses food spoilage, techniques of maintaining a consistent acceptable quality, methods of distribution to insure that the maximum quantity of acceptable food material reaches the consumer and help to apply better techniques to existing systems of fish handling. Such an approach, I feel, would be most successful if the knowledge is imparted at some basic level to the fishermen. Unfortunately, most of the students on return to their home countries are immediately promoted from the field to an office job. Of course they are very happy about this — office jobs usually are more attractive than getting your feet dirty in the field. And this brings me to the point which I would like to make Mankind seems to take status as being an important aspect of life. Nobody can stop the laboratory cleaner dreaming about being the managing director. In the UK food industry, we call the laboratory cleaner a hygiene officer and he is paid as much as some senior production men. Perhaps in fact we may have to rename this job or the identification of the man who is getting his feet dirty using technology in the field and pay him as much as a director of research organization. Thank you Sir.

Joseph H. Hulse

Thank you MR. DEBLING. Could I now call on DR. KOSIKOWSKI of Cornell University.

Frank Kosikowski

I would like to speak a little bit about our program at Cornell University.

Some years ago I spent some time as a technical officer of the FAO. This activity enabled me to realize, after my return to university that things that happened through the FAO and things that we teach at the university are quite different subjects. So I decided when I came back in late 1963 to initiate a course on international food development at Cornell University which later has developed into a major. In our graduate field of food science and technology we have six majors. One of these is now called international food science, and it developed really from this rather basic interest of mine in trying to get to our students the realities of international food science. Now in this particular program we have nothing special, it goes through our regular academic area. Cornell University was very receptive to this initiative, and since its starting in 1963 it has gone through various modifications. I would like to describe briefly what has happened since that year. Our main objective is to try to provide leaders and leadership in the field of international food science and development; it is not to develop technicians but to try to develop people who can understand the basic overall problem that faces us throughout the world. But initially, and here is the difference, they are not economists, philosophers or law

degreed people; they are food technologists. We must insist that they have a basic structure in food technology before they can take this major. So our requirements, in the light of the graduate field of Cornell University, follows the same requirements in the field. People must satisfy a committee, must take various courses, must pass an examination, and we have found over the past eleven years that this major has attracted a large number of excellent highly averaged students. I can say, having worked with them very closely, that this type of student is exceptional. 25 per cent of our students are from USA and the remaining 75 per cent from foreign countries such as Nigeria, Chile, India, Mexico, Haiti, Tanzania. With this kind of mixture we have a very good and very active performance in many ways. We have not insisted on some formal course requirements, but the committees that are involved with these students may ask them to first take formal courses in food science which they lack and then take several courses given by our department of food science on high protein food technology, on fermentation, on food dehydration. In a final year they may be required to go to an international law school; they may go to an international school of sociology, anthropology, etc. All this is integrated in the frame of studies. But each student, of course, is treated differently. In the practical field we have our students working at laboratories. Most of the students are financed by FAO, AID, Ford Foundation or the countries from where they come. So here is the connection between what I am saying and what objectives of this program are.

We are working very hard to give technical information to the students, but I think the most important aspect of this program is that the food science students acquire a global knowledge on the problems of food development. Too many students in food science and technology cannot see beyond the text. We have to try to educate a certain type of students to understand the problems on information interchange. So that in our program we insist that students be able to view the broad problems of food development on a worldwide scale. Our students, in that respect, are returning to their countries, I think, in a very highly centered manner. We think that, in the future, these students will become leaders for technical and research institutes, talked about this morning.

Joseph H. Hulse

DR. KOSIKOWSKI was the last who asked to speak. There are a number of questions and not so much time as would be desired. The first question has been addressed to DR. PÉREZ VILLASEÑOR from Mexico.

Javier Pérez Villaseñor

DR. PARPIA asked what has been done to set up a nucleus for food technology development in Mexico and what remains to be done. Both questions are really not easy to answer because they require lengthy replies. However, I shall try to answer them as briefly as possible.

First of all, what has been done is the following. There are several groups or nuclei throughout the country related to food science and technology. They are mainly at the universities teaching and doing some research. The staff is small, and therefore the feed-back is rather limited. There is the need, consequently, to train more people in areas which have not been touched so far and in which trained personnel is urgently needed. The standard of training already in existence must be upgraded. This would need training of the trainer first. They either can be sent to appropriate centres abroad, or courses must be set up in order to impart specific knowledge required. In general, the institutions doing teaching and research need to improve their working conditions and facilities; they should set up well equipped laboratories, not fancily equipped, but with the equipment necessary to carry on the work. Most of them have no pilot plant facilities which I consider to be absolutely necessary for the training of people who are going to work in industry. And as I mentioned, laboratory facilities are almost inexistent or are very limited. Let us put it this way, there are many shortcomings, and

efforts should be concentrated on overcoming them. Above all, I feel that some coordination of efforts is necessary, perhaps through setting up a National Institute, or whatever name you may give to such a centre, to carry on the job on food science and technology at a national level.

Perhaps that answers DR. PARPIA's question.

Joseph H. Hulse

Thank you very much MR. PÉREZ VILLASEÑOR.

I would like to invite our friends from Africa, Asia, Latin America and the Near East to speak about the main characteristics of joint companies in LDC contributing usefully to the local economies. I mean joint companies between those of the developed world and those of the less developed world. I wonder if there are any of our friends from the developing countries who care to cite instances of successful joint efforts and perhaps indicate to us why these joint efforts were successful in the lines of their own economy.

Isaac Akinrele

We have in Nigeria a number of highly successful commercial enterprises which are partly owned by the Government, and they have achieved a very large success by producing good foods from adequate raw materials making use of a very competent management. My main objection is that most of the raw materials are actually imported. The success of companies from the United Kingdom and Western Europe is based on raw materials which they import. It is my opinion that besides good management the success of such joint companies should be judged by their ability to do a complete job using indigenous raw materials to manufacture a good food product. Only then can they bring real benefit to the population of the country as a whole.

Joseph H. Hulse

Thank you very much. Are there any other people, particularly from the developing countries of Africa, Asia, Latin America or the Near East, who would like to speak on this subject.

Mario Solorzano Marín

My name is SOLORZANO, and I am from Nicaragua. Our country, like many other countries from Central America, is facing problems. As soon as any national company starts to be successful, it is taken over by some foreign company. Our biggest industries, meat, oil, corn and some others, after achieving good success in the local market cannot survive and are finally bought over. They prove to be successful in the joint venture mostly, of course, for the benefit of owners and the licensees. So we are in a period of not being able to develop a true national industry because of the outside pressures on our economy.

Joseph H. Hulse

Thank you very much. I think we can have one more comment on this.

MR. ROSENBERG has asked a question to DR. PARPIA, but he asked me to answer it. The question is the following, DR. SPENSLEY has spoken about the necessity that a research centre should form an integral part of the development. I do agree with this. What does it mean to form an integral part of the development? I really have some doubts in the case of industries having the control over it. Are they not often more concerned with their own development than about the development of their country? And would it not be more appropriate that the institutes are part of the Ministries who are more directly

concerned with the need for national development and are aware of the need for technological changes?

I shall try to answer this question. Let me say that in developing countries as well as in developed countries the major research institutions are usually government sponsored. If we consider the particular case of developing countries, we should recognize the difference between the types of institutions. A university, for instance, and the institutions for applied research, both being autonomous but sponsored by the government. The major objective of a university is to build professional competence in men and that of the research institutes to solve specific problems of scientific and technological interest to the economy. The difference is that the institutes, considering their organizational function and funding, have another vitally important role of advising the government and industry on matters of policies in the field of science and technology. Their results of their research should be available to all industries. It is also clear that to fulfill its objectives, the research institutions must work in close cooperation with the food industry. Well, for instance, an area is considered strategic, institutes should give priority to developments in it. When the results are obtained they can be patented an can be made available to all interested industries by the government.

Joseph H. Hulse

I have a question from DR. GORGATTI NETTO directed to me. He said that I spoke of the cultural aspects which influence the absorption of the new technology. Is the difference in salaries one of these cultural aspects? What is the role of this in developing food industries?

Of course, the difference in wages is an important point to be considered, but this is not the major issue. What I was trying to say is that professionals from developed countries who are invited to work on research problems in developing countries have to find solutions to these problems according to the approach that they have been using in their own countries, and in many cases this aproach may not be the most feasible under the social and economic conditions prevailing in the developing countries.

Thank you.

M. Austin

.....whatever else could I say, if you point out that the urban production might be less beneficial specially by the multinational corporations, then, is it not socially more profitable to look for a wide production base where more people can get employment and where you have got a base for the development of the country? Does not the government have to give support to this type development? That is what I mean with it.

K.T. Achaya

I think you have really amplified what I said that the decisions are difficult ones. It is true that you reach different segments or the population both ways. There is unfortunately a demand from the urban elite for better quality products, but, on the other hand, the other sector, the less economically able, has a very important role to play. These are factors on which decisions must be taken and sometimes they are rather difficult ones.

Unknown speaker

I would like to make some comments on something that has come up several times today, and I think it is very much related to this particular question.

Foods have an unfortunate property of being highly perishable, and in many instances, the first preservation takes place quite a long way from the point of harvest. The primary concern of food industries in developing countries, as Dr. Parpia indicated earlier, may very well be to have to reduce this 20 per cent or 40 per cent loss, whether

an industry operates profitably by conventional economics or not. I would like to have comments from others in relation to placing the food processing close to the point of harvest where the deterioration begins. This is an important aspect of rural industry; perhaps the rural food processing industry may become more important due to the very critical world food situation we are facing at the moment.

K.T. Achaya

The products manufactured by large companies usually go the the urban centres with some penetration in terms of the market approach into the rural areas; while the products made in rural areas have usually a small turnover which provide a limited profit, but provide enough income to the poor people to survive and to live on.

There have, therefore, to be distinctions, for example, in the distribution and use of raw materials for processings. I will give you an excellent example not from the food industry but from something fairly close to it, the soap industry. We import into my country, India, a lot of tallow at a low prices. This tallow goes to the large mechanised soap industry which make high quality soap. The distribution of such low cost raw materials to rural industries presents a cost problem. Therefore political and economic decisions have to be taken in such matters and there is a constant stream of complaints from the rural industry that it gets an unfair deal.

This problem could arise in the food industry, but to a lesser extent, because few food materials are really not imported except some basic ones. But this involves difficult decisions.

Would you subsidize inefficient production or provide the raw materials at the cost, perhaps, for the benefit of the rural consumer? Here the decision becomes political and has sometimes to be taken.

L. Glenn

One may be, at least, indebted to rural areas. The problem is a very simple one of economics and this is directly tied to the villages, to the farmer. When you want to mechanize traditional rural industries, the amount of capital available is so small that you have to find low investment technologies. I think the most profitable idea is to ruralize the industry through such efforts.

Unknown speaker

I wish to make a coment.

It is a matter of development of certain appropriate technologies even for temporary conservation of foods. This is not for rice as such but also for other crops such as grund nuts.

H.A.B. Parpia

The discussion on the subject of technology transfer has indeed been stimulating and the views expressed by the Distinguished Members of the Panel and the Participants from the audience have been most valuable. There is distinct recognition of the nature and magnitude of the food problem as it exists today, and the proportions it can assume. It is clear that a well managed use of science and technology can find a solution but it is recognized that this would need a concerted action. One of the most effective ways to improve food supplies, would be through the selection, transfer and utilization of appropriate technologies should be economically sound to justify investment.

The emphasis on building competence at national level to select and transfer technologies and establishment of inter-country cooperation through networks of institutions at sub-regional, regional and global level for this purpose has been recognized. Efforts are needed on the part of the UN agencies, especially FAO, the professional societies of

Food Scientists and Technologists, particularly IUFoST to take action for building mechanisms that would speed up transfer of appropriate technologies between developing countries, from advanced to developing countries and from research and development institutions to industry.

At present less than 2 per cent of the research and development budget of the world is invested in the developing countries. It is therefore essential that the governments and industry in developing countries increase their contribution to the efforts for technology transfer, research and development in order to achieve greater self-reliance to accelerate economic development.

Only through a conscious effort can the errors of the past in technology transfer be avoived and technological self-reliance be built for establishing indigenously owned food conservation and processing industries in developing countries. The appropriate technologies to be selected and developed must be economically sound, capital saving, labour intensive, scientifically modern and poverty reducing.

The developing countries can ill afford to work on hobby oriented research. The problems on which they work must be well identified to indicate the impact which their solution would have on improving food supplies and accelerating developing.

In assisting developing countries IUFoST members have a special role to play. Through the international body of professional scientists we can help to build a policy, philosophy and a programme of action to transfer and develop appropriate technologies that can overcome hunger and malnutrition.

With these few words, Ladies and Gentlemen, I want to express my gratitude first to the members of the panel and then on their behalf and on my own behalf to all of you for your most valuable contributions.

Round-Table 3

RECENT ACHIEVEMENTS IN THE UTILIZATION OF PROTEIN FROM DIFFERENT SOURCES

FIRST PART: SINGLE CELL PROTEIN
SECOND PART: LEAF PROTEIN

Chaired by

N. S. Scrimshaw

Proc. IV Int. Congress Food Sci. and Technol. (1974) Vol. V, p. 453 – 457

FIRST PART

SUMMARY OF THE WORKSHOP ON SINGLE CELL PROTEIN

This workshop was attended by approximately twenty-five persons, lasted for two and one-half hours, and covered the following topics:

1. Scope

It was agreed that food scientists and technologists should consider the whole range of single cell protein materials for both animal and human feeding. These include yeast, bacteria and filamentous microfungi. Leaf protein were considered in a separate workshop. It is also necessary to be concerned with a wide variety of substrates. Traditional substrates have been carbohydrate, such as molasses and starch, and waste products such as the sulfite liquor of the paper industry. There is now much interest in petroleum hydrocarbon substrates, including methanol, ethanol, methane, gas-oil, normal paraffins, and normal alkanes. The workshop emphasized that all of these are relatively expensive for the developing countries, and some use non-renewable resources. More research was urged to develop feasible ways of producing single cell protein material on cellulosic waste products such as bagasse cereal hulls, and coffee pulp.

2. Nomenclature

Even though not precisely descriptive, since in a sense all protein comes from single cells, the name single cell protein was chosen for a conference in 1972 as a neutral term in order to avoid names with a prejudicial connotation, such as microbial proteins or the unfortunate term petroleum protein, which has led to consumer protests and become a political issue in Japan. While the name single cell protein seems to be serving its intended purpose, it is important to recognize and emphasize that there are many different types of single cell proteins. Some of them are never intended to be suitable for human feeding, while others may be products of good food quality. As single cell proteins come on to the market, they will need to be identified by generic and trade names.

3. Organisms

As in the case of higher plants, out of the wide range of microorganisms available, some will be quite safe and suitable for human consumption and the majority probably toxic or otherwise unacceptable in some manner. In between will be a range of organisms that can be rendered suitable by appropriate processing, but for which the processing conditions will be important if the material is to be used in food. Parallels with foods from higher plants are the processing of bitter cassava and the cooking of beans. In short, organisms must be carefully selected and processed for safety and tolerance.

4. Testing for animal use

Products that are toxic to animals or that do not promote good growth automatically will be eliminated from practical use. Therefore, the objective of testing materials intended only for animal feed is to insure that the resulting animal products are safe

as they are fed to man. This will be difficult to prove directly, and a great deal of common sense is needed. There is a danger of excessive regulatory requirements for the use of SCP in animal feeds not required of other feed ingredients. In fact, animals are now fed, without regulations, all kinds of waste materials with far greater potential hazard than the single cell protein materials currently proposed for animal feeding. Considerable education of the public politicians and regulatory agency personnel is likely to be required. Following a working group meeting in December 1973, on guidelines for the development of single cell protein for animal feeding, PAG guideline No. 15 on this subject is now available from the Director of the Secretariat, Protein Advisory Group, Suite A-606, Alcoa Building, 866 United Nations Plaza, New York, New York 10017.

5. Testing for human use

When an SCP is intended for human use, extensive key clinical evaluation is required, including short-term toxicity studies in at least two species of animals, long-term trials, and in some cases, specific evaluations for possible mutagenic or teratogenic effects. These are included in PAG guideline No. 7, also available without charge as listed above.

It was strongly emphasized that completely satisfactory pre-clinical trials do not eliminate the necessity of careful and systematic human studies—even sub-human primates cannot be substituted. There are two reasons for this. One is that the product may not be palatable or may cause gastrointestinal discomfort that is not apparent in the animal trials. The other and more serious reason is that many SCP materials apparently can cause the development of gastrointestinal allergic responses in a significant number of sensitive subjects. In such a case, the offending substance must be eliminated by processing or the plan to use the material for human feeding abandoned.

A characteristic of all SCPs, because they must be produced by rapidly growing cells in order to be feasible, is a relatively high content of nucleic acid per cell. Nucleic acid in the human body is converted finally to uric acid. All other animals, except man and the higher apes, have an enzyme, uricase, which breaks down uric acid to the more soluble allantoin. Without this enzyme, uric acid levels may rise in the blood to the point where uric acid crystals are deposited in the joints to cause gout, or they may be precipitated in the kidneys to cause stones. Present recommendations are that the amount of nucleic acid added to the diet in single cell protein should not exceed 2 grams per day for an adult and proportionately less by weight for children. Since this corresponds to roughly 20 grams of yeast and slightly over 10 grams of bacterium, it is evident that nucleic acid levels must be reduced if single cell protein is to serve as a major protein source in human diets. The 2-gram limit is now undergoing review and could be lowered.

6. Special problems

In the testing of certain single-cell protein materials in experimental animals, neurocalcinosis has sometimes been observed. It has also been seen in some tests of fish protein concentrate and certain oil seed protein concentrates. Recent studies suggest that this may be due to a combination of mineral imbalance, a low level of phosphorous relative to calcium and potassium, and excessive addition of methionine. It appears when the material is fed at the highest practical levels, and is easily correctable.

Ordinary food yeast, *Candida utilus,* grown on sulfite liquor, is extensively used in processed foods in small quantities as a vitamin source and is on the United States list of food additives generally recognized as safe. However, when this material has been fed at high levels, such as would be required if it were to be a major protein source, it has caused skin reactions in some individuals. It is important to recognize, therefore,

that satisfactory experience even over many years with low levels of a food additive may not indicate suitability for use of the same material at high levels.

It was also noted that the amounts of heat given off during fermentation are often not recognized. There are serious problems of dissipating this heat in hot tropical countries and this makes SCP production more costly, even if thermophilic organisms can be used. Without thermophilic organisms, SCP production in hot locations may be impractical.

7. Economics

In 1973, the high soybean meal prices and scarcity of fish meal made the production of SCP as a protein source in animal feeds using petroleum hydrocarbons as an energy source seem economically feasible. Recent increases in petroleum prices have worsened the commercial prospects of SCP for animal feed in Europe using this energy source. The current economic situation and growing concern for the use of renewable resources justifies greater emphasis on the use of vegetable wastes as substrates for SCP production for animal feeding. SCP production for human feeding using carbohydrate substrates such as starch could still be commercially attractive if the product had useful functional properties as well as protein value.

The discussion strongly emphasized that SCP produced for animal feeding on purified substrates, or any commercially produced SCP for human consumption, was likely to be uneconomical and impractical for less-developed countries under present conditions. The product would simply be too costly relative to other protein sources to find a market. For this reason, less developed countries should be discouraged from investment in large scale single cell protein production activities because there will be no market for the product at realistic prices. Some developing countries are currently negotiating for outside technical assistance in the building of single cell protein plants for political and emotional reasons without a thorough understanding of the economic and market realities.

8. Recommendations

The principal recommendations that emerged from the discussion were:

a) increased attention should be given to research for the production of SCP for animal feed on cellulosic and other wastes;

b) there should be greater recognition of the need for extensive pre-clinical and clinical tests and for processing to reduce RNA and possibly other undesirable factors for any SCP proposed for human consumption;

c) feed-grade SCP should not be held to food grade standards; and, the standards for SCP used for animal feeding should not be different in principle from those for any other component of animal feeds;

d) governments should be discouraged from investing in uneconomic SCP production facilities for emotional or prestige reasons.

Reviews

Mateles, R. I. and Tannenbaum, S. R. (Eds.), *Single-Cell Protein.* Cambridge, The M.I.T. Press, 1968.

Gounelle de Pontanel, H. G. (Ed.), *Proteins From Hydrocarbons.* New York, Academic Press, 1972.

Tannenbaum, S. R., Wang, D. I., and Scrimshaw, N. S. (Eds.). *Single-Cell Protein II.* Cambridge, Massachusetts. The M. I. T. Press, in press.

SECOND PART

LEAF PROTEIN – DISCUSSION

The Chairman opened the discussion by drawing the attention of those present to the International Biological Programme Handbook No. 20 on Leaf Protein, published in 1971 which summarised all aspects of research prior to that date very satisfactorily. Work was now continuing in a number of countries, which was aimed at exploring ways of improving yields, extending the numbers of useful plant species, carrying out further animal tests and human clinical trials and attempting to improve the process economically For the benefit of those present at the Workshop he then outlined the papers submitted for presentation at the meeting, the majority of which had not in fact been delivered. With reference to the paper of Bickoff and Kohler on the commercial production of leaf protein in which reference had been made to research in progress designed to yield a pigment-free palatable product, DR. SAUNDERS of the U.S.D.A. Western Regional Laboratory described the process which allowed the separation of an almost-white fraction after the bulk of the green chloroplastic protein had been precipitated and separated. He emphasised that the U.S.D.A. process was essentially a "scalping" process which was not aimed at giving the maximum possible yield of protein.

The question of toxic effects of LPC was raised and it was generally agreed that sufficient animal testing as well as human clinical trials had now been carried out to indicate that there was no danger from properly prepared samples. Such toxins as alkaloids and saponins would ordinarily be left in the aqueous liquor after precipitation of the protein but thorough washing of the protein was desirable for material destined for human consumption. DR. MILNER (U.N. Protein-Calorie Advisory Group) queried the feasibility of planting crops solely for the production of leaf protein. Dr. WOODHAM thought that although this did not seem to be economically sound at present, the time might well come when this approach would be feasible. At present the harvesting of wild plants on a sufficient scale could only be done in limited areas where labour was cheap and the natural vegetation was of a type which could be harvested easily. Tree leaves provided obvious difficulties. He drew attention however to the work now being carried out in the U.K. on the direct feeding of grass juice to pigs. This had been shown to be both technologically and economically feasible on a comparatively small scale but there were chemical engineering problems with regard to large-scale feeding. Slurries that were adequately mobile were also too dilute to contribute a satisfactory quantity of protein. Dr. DE OLIVEIRA (Lisbon) described a process which he claimed could surmount this problem. His process involved fermentation of the juice followed by distillation. Dr. MILNER mentioned the commercial process developed in Hungary, largely based on the original Pirie process and this, he said, was operating satisfactorily at present.

MR. ROLF CARLSSON (University of Lund) showed some slides which indicated the advantages of members of the *Chenopodium* species as sources of leaf protein. *Chenopodium* is a prolific crop and the cells are easily ruptured giving good yields of protein. He referred in passing to the work of CHRISTIAN TRÄGARDH (University of Lund) who has compared methods for concentrating the raw juice, including evaporation and ultrafiltration. This worker had used isopropanol successfully for decolourising and defatting the protein extracts.

DR. KOHLMANN (Brazil) spoke of the economies of leaf protein production and said that even if leaves were obtained at no cost the final product was still more expensive than the current cost of soyabean meal. Various representatives of industry who were present disagreed with this statement.

In conclusion the Chairman referred to work now in progress in the U.K. on the attempted establishment of a completely integrated process which would allow the use of all parts of the plant. PIRIE has said that to be economic a process must utilise the fibrous residue as well as the liquors remaining after separation of the protein. Micro-organisms have been grown successfully on the liquors and the fibrous residue has been fed to sheep and cattle in New Zealand. Information is now needed on the yields of primary protein, microbiological protein and roughage from different plant species in relation to the area of land required to produce the crop.

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